



**EPIDEMIOLOGY AND EFFECT ON BIRTHWEIGHT OF
PRENATAL ZINC AND VITAMIN A DEFICIENCIES IN RURAL
SIDAMA, SOUTHERN ETHIOPIA**

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**PhD DISSERTATION FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (PhD)**

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**ADDIS ABABA UNIVERSITY
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SIDAMA, SOUTHERN ETHIOPIA**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF GRADUATE
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DISSERTATION APPROVAL

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Original Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. Gebremedhin S, Enquesslassie F, Umata M. Prevalence of prenatal zinc deficiency and its association with socio-demographic, dietary and health care related factors in rural Sidama, Southern Ethiopia: A cross-sectional study. *BMC Public Health* 2011;11:898 doi:10.1186/1471-2458-11-898.
- II. Gebremedhin S. and Enquesslassie F, Umata M. Prevalence and correlates of prenatal Vitamin A deficiency in rural Sidama, Southern Ethiopia. (Under review, *Journal of Health Population and Nutrition*)
- III. Gebremedhin S, Enquesslassie F, Umata M. Effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama, Southern Ethiopia, (Under review, *BMC Pregnancy and Child Birth*)
- IV. Gebreselassie SG, Gashe FE. A Systematic review of the effect of prenatal zinc supplementation on birthweight: Meta-analysis of 17 randomized controlled trials. *Journal of Health, Population and Nutrition* 2011; 29(2):134-140.

Acronyms and Abbreviations

AAS	Atomic Absorption Spectrometer
AAU	Addis Ababa University
AIDS	Acquired Immuno Deficiency Syndrome
ANC	Antenatal Care
ANOVA	Analysis of Variance
AOR	Adjusted Odds Ratio
ARR	Adjusted Relative Risk
ASL	Above Sea Level
BMI	Body Mass Index
CDC	Center for Disease Prevention and Control
CEB	Child Ever Born
CED	Chronic Energy Deficiency
CHPs	Community Health Promoters
CI	Confidence Interval
CIC	Conjunctival Impression Cytology
CRP	C-Reactive Protein
COR	Crude Odds Ratio
CRR	Crude Relative Risk
CSA	Central Statistical Agency
DALY	Disability Adjusted Life Years
DD	Dietary Diversity
DDS	Dietary Diversity Score
DHS	Demographic Heath Survey
DL	DerSimonian and Laird's
DNA	Deoxyribonucleic acid
EDHS	Ethiopia Demographic Heath Survey
EHNRI	Ethiopian Health and Nutrition Research Institute
ES	Effect Size
FANTA	Food and Nutrition Technical Assistance
Fe-Fol	Iron and Folate
GDP	Gross Domestic Product
GPS	Global Positioning System
HDDS	Household Dietary Diversity Score
HEWs	Health Extension Workers
HFIAS	Household Food Insecurity Access Scale
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IDD	Iodine Deficiency Disorder
IGA	Income Generating Activity
IQ	Intelligence Quotient
IQR	Inter Quartile Range

IRB	Institutional Review Board
IU	International Unit
IVACG	International Vitamin A Consultative Group
IZiNCG	International Zinc Nutrition Consultative Group
IUGR	Intra Uterine Growth Retardation
Kcal	Kilocalorie
Kg	Kilogram
KMO	Kaiser-Meyer-Olkin
LB	Live Births
LBW	Low Birthweight
LNMP	Last Normal Menstrual Period
MAL	Meta-analysis based on Literature
MEDLINE	Medical Literature Analysis and Retrieval System
MUAC	Middle Upper Arm Circumference
NGOs	Nongovernmental Organizations
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
PCA	Principal Component Analysis
PEM	Protein Energy Malnutrition
PI	Principal Investigator
PIH	Pregnancy Induced Hypertension
PNC	Postnatal Care
PPM	Parts Per Million
PROM	Premature Rupture of Membrane
RBP	Retinol Binding Protein
RCTs	Randomized Controlled Trials
RDA	Recommended Daily Allowance
RDI	Recommended Dietary Intake
RDR	Relative Dose Response
RPM	Rotation Per Minute
RR	Relative Risk
RSD	Relative Standard Deviation
SAM	Severe Acute Malnutrition
SD	Standard Deviation
SI	Synergy Index
SNNPRS	Southern Nations, Nationalities, and Peoples Regional State
SPSS	Statistical Package for the Social Sciences
UNDP	United Nations Development Program
UNICEF	United Nations Children's Fund
VA	Vitamin A
VAD	Vitamin A Deficiency
VAS	Vitamin A Supplementation
VCHPs	Voluntary Community Health Promoters
VIF	Variance Inflation Factor
WHO	World Health Organization
ZD	Zinc Deficiency

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I. Summary

Background:

In the developing world pregnant women face diverse nutritional deficiencies with potentially grave consequences. The most prevalent deficiencies are assumed to be Protein Energy Malnutrition (PEM), Iron Deficiency Anemia (IDA), Vitamin A (VA) Deficiency (D), Iodine Deficiency Disorders (IDD) and Zinc Deficiency (ZD). Nevertheless, currently limited information is available about the prevalence of prenatal zinc and vitamin A deficiencies in Ethiopia. Previous studies which attempted to identify the correlates of the deficiencies and their effects on birthweight ended up in equivocal conclusions.

Objective:

To assess the prevalence, correlates and effect on birthweight of prenatal ZD and VAD in rural Sidama, Southern Ethiopia.

Methods:

The study included a community based cross-sectional baseline study to assess the prevalence and correlates of the deficiencies and a prospective cohort study to evaluate the effects of the deficiencies on birthweight. The baseline study was conducted in January 2011 among 700 randomly selected pregnant women. Data on potential correlates of the deficiencies were gathered using a structured questionnaire. Serum zinc, retinol, ferritin, hemoglobin and C - Reactive Protein (CRP) concentrations were determined from venous blood following standard procedures. In the cohort study 575 pregnant women who were in their second or third trimester during the baseline survey were successfully followed until delivery and birthweight was measured within 72 hours of birth. Data were analyzed using linear, logistic and log-binomial regression models. The dissertation also incorporated a meta-analysis of Randomized Control Trials (RCTs) so as to assess the effect of prenatal zinc supplementation on birthweight. Relevant studies were identified through web-based search. Effect Size (ES) was measured based on standardized mean difference and pooled using a variant of random effect model.

Results:

About 53.0%, 37.9% and 17.4% of the subjects had ZD, VAD and Iron Deficiency (ID). Taking the three deficiencies into consideration, 32.9% of the subjects had two or more concomitant deficiencies and 5.1% had three of the deficiencies.

Elevated CRP and gestational age were significant negative correlates of zinc status. ZD was substantially higher among pregnant women from food insecure households and amongst those who had low Dietary Diversity Score (DDS) in the preceding day of the survey. Illiterates and women devoid of self income had 1.71 (95% CI: 1.09-2.60) and 1.74 (95% CI: 1.11-2.74) times increased risk of ZD. The risk was also 1.65 (95% CI: 1.02-2.67) times higher among women from maize staple diet category compared to *Enset*. Women aged 25-34 and 35-49 years were 1.57 (95% CI: 1.04-2.34) and 2.18 (95% CI: 1.25-3.63) times more likely to be deficient than those aged 15-24 years. Grand multiparas were 1.74 (95% CI: 1.09-3.23) times at risk than nulliparas. Frequency of coffee intake was negatively associated to zinc status. Positive association was noted between serum zinc and hemoglobin concentrations.

Elevated CRP was associated with 22.5% reduction in serum retinol concentration. Women at their third trimester had 2.59 (95% CI: 1.23-5.48) times increased risk of VAD compared to those at the first trimester. The risk of VAD was significantly higher among illiterates and women without their own income. Women aged 35-49 years had 2.23 (95% CI: 1.31-3.81) times higher risk compared to those aged 15-24 years. Women with low DDS were 1.94 (95% CI: 1.17-3.19) times more likely to be deficient than their counterparts with high DDS. Compared to nulliparas, multiparas had 2.25 (95% CI: 1.20-4.22) times increased risk of VAD. VAD and ZD were associated to each other with adjusted OR of 1.80 (95% CI: 1.28-2.53).

The mean birthweight among babies born to women who were at their second or third trimester at the time of exposure assessment was 2896 g and 16.5% (95% CI: 13.5-19.6%) had LBW. Prenatal ZD and VAD were not significantly associated to LBW with Adjusted Relative Risk (ARR) of 1.25 (95% CI: 0.86-1.82) and 1.27 (95% CI: 0.86-1.87), respectively. The occurrences of ZD and VAD, neither in the second nor third trimester, were associated to LBW. The deficiencies did not show synergetic interaction in causing LBW with SI of 1.04 (95% CI: 0.17-6.28). Significant determinants of LBW were maternal illiteracy, maternal thinness and stunting, primiparity, female sex of the baby and elevated CRP during pregnancy.

Among 17 RCTs included in the meta-analysis, 3 reported positive association between zinc supplementation and birthweight, 1 had marginally negative association where as 13 found no association. Based on DerSimonian and Laird's random effect model, the pooled ES was 0.071 (95% CI: 0.162 to -0.019) and it remained insignificant after stratification was made based on the dose of supplementation (optimal or high dose), design of the studies (community or health institution-based), and development status of the study country (developed or developing).

Conclusion:

ZD and VAD are of public health concern in the area. Key correlates of ZD were household food insecurity, low DDS, dependency on maize as a staple diet and low level of consumption of animal source foods. Illiterates and women devoid of self income had increased risk of ZD. Grand multiparity, old age pregnancy and frequent consumption of coffee were negative correlates. Pertaining to VAD, advanced gestational age elevated CRP were negatively associated with serum retinol level. Advanced maternal age, inferior socio-economic status, dependence on poorly diversified and plant based diet, ZD and history of too close and too many births were pertinent correlates of VAD. Prenatal ZD and VAD occurring neither in the second nor third trimester were not associated to LBW. Further, the deficiencies did not show synergetic interaction in causing LBW. Based on the meta-analysis, prenatal zinc supplementation did not show positive effect on birthweight.

Recommendation:

Zinc and VA deficiencies should be combated through food-based approach as it is a sustainable strategy to prevent multiple micronutrient deficiencies. Approaches that focus on dietary diversification like backyard gardening and poultry production should be promoted. The existing efforts to improve women's awareness about optimal nutrition prior and during pregnancy should be strengthened through building the capacity of health extension workers and voluntary community health promoters. Strong intersectoral collaboration must be established between the health and agriculture sectors so as to address the root causes of malnutrition. Use of household based phytate reduction techniques, utilization of zinc containing fertilizers should be considered as potential strategies to combat ZD. Expansion of family planning coverage, livelihood promotion and socio-economic empowerment of women shall have affirmative influence.

Key Words:

Prenatal zinc deficiency, prenatal vitamin A deficiency, low birthweight.

II. Introduction

Background

Women throughout their lifecycle are vulnerable to macro- and micro-nutrient deficiencies emanating from multitudinal factors. Besides the vulnerability posed on the general population by the root causes of malnutrition, women encounter elevated risk due to physiological, reproductive, medical, socio-cultural and economical factors (1).

Pregnancy is the most nutritionally demanding period in a woman's life. Optimal nutrition is indispensable to support the growth of fetus and placenta, and to accommodate physiological changes of pregnancy. Accordingly, except for sodium and potassium, the Recommended Dietary Allowance (RDA) of all essential macronutrients, vitamins and minerals is raised during pregnancy. If the increased demand is not adequately satisfied, maternal stores will be depleted and the risk of malnutrition and its consequences will mount (2).

Especially in the developing countries like Ethiopia, pregnant women face diverse nutritional deficiencies with potentially grave consequences. The most prevalent nutritional deficiencies are assumed to be Protein Energy Malnutrition (PEM), Iron Deficiency Anemia (IDA), Vitamin A Deficiency (VAD) and Iodine Deficiency Disorders (IDD). Recent studies also recognized Zinc Deficiency (ZD) as an emerging public health threat (3).

According to Ethiopia Demographic and Health Survey (EDHS) 2005, 26.5% of women of reproductive age had Chronic Energy Deficiency (CED), 6.1% experienced night-blindness during their recent pregnancy and 30.6% of pregnant women were anemic (4). A study in 2005 also found that more than 30% of women had severe iodine deficiency (5). So far the national prevalence of ZD has not been investigated. Nevertheless, a study in rural district of Southern Nations Nationalities and Peoples Region (SNNPR) witnessed its public health significance (6).

Thus the current study is dedicated to assess the magnitude and correlates prenatal zinc and vitamin A (VA) deficiencies and their effect on birthweight in rural Sidama, Southern Ethiopia.

Problem Statement

ZD is widespread public health problem in the world (7). According to WHO, it affects about a third of the world's population. In 2004 International Zinc Nutrition Consultative Group (IZiNCG) estimated that 21.1% of the Ethiopian population was at risk of inadequate dietary zinc intake (7). However empirical and updated evidence on the prevalence of ZD amongst women of reproductive age, especially pregnant women, are sparse. A study among pregnant women in rural Sidama, SNNPR, reported 72% prevalence of ZD and 99% prevalence of inadequate dietary zinc intake (6); whereas, a study among lactating women in Addis Ababa found merely 11.3% with marginal zinc deficiency (8).

Studies conducted over decades consistently indicated the public health significance of VAD in Ethiopia (9-15). Nonetheless, as nearly all of the studies were conducted in preschool children and women of reproductive age and as most of the available studies in pregnant women were principally reliant on clinical deficiency indicators, the full spectrum of the problem in pregnant women has not been revealed. Though WHO estimated 13.2% prevalence of sub-clinical VAD in pregnant women of Ethiopia (16), the available three studies reported higher figures ranging from 17 to 27% (17-19).

Several studies associated ZD and VAD with various adverse pregnancy outcomes. ZD has been linked with Pregnancy Induced Hypertension (PIH), Premature Rupture of Membrane (PROM), placental abruption, prolonged labor, hemorrhage, infections, Intrauterine Growth Retardation (IUGR), congenital anomalies, neonatal morbidity and poor neurobehavioral development (20). VAD has also been related with anemia (21, 22), prematurity (22-25), IUGR (24, 26), malformations (27) infections (28), PIH (28-31), vertical transmission of HIV (32), poor infant growth (21), infant mortality (21, 33) and maternal mortality (33). Nevertheless most of the aforementioned consequences are still contentious.

LBW is the single most important predictor neonatal survival and a significant determinant of post-neonatal infant mortality and childhood morbidity (34). It has been estimated that LBW babies are approximately 20 times more likely to die than normal babies (34). However, regardless of the potential harmful contribution of prenatal VA and zinc deficiencies to LBW, their independent effect is still contentious and their potential interaction has not been studied. Randomized Controlled Trials (RCTs) which evaluated the effect maternal zinc supplementation on birthweight yielded contradictory conclusions (35). Reasonable numbers of observational studies have found positive relationships between maternal zinc status and birthweight (36). However, they had limited sample sizes or failed to adequately control confounders. Pertaining to VA, observational studies in Israel (37), UK (25) and Guatemala (26) witnessed positive relation; whereas, studies in Bangladesh (38) and USA (39) found no association.

In consideration of the clinical consequences and public health significance of prenatal zinc and VA deficiencies, many studies in the developed and developing world attempted to identify their potential risk factors including a range of dietary, socio-cultural, economic, reproductive and medical factors. However, their findings were inconclusive. For instance the effects of socio-economic status, maternal age, gestational duration, parity, birth interval, iron supplementation, maternal workload, maternity service utilization etc on prenatal VA or zinc status are controversial.

Accordingly, this study was conducted in rural Sidama zone, Southern Ethiopia with the purpose of assessing the prevalence, correlates and effects on birthweight of prenatal zinc and VA deficiencies. The setting was selected as an earlier study (18) in the locality with limited sample size and geographical coverage reported exceedingly high prevalence of prenatal zinc and VA deficiencies.

Background of the Study Area

Ethiopia is situated in the horn of Africa bounded by North Sudan, South Sudan, Kenya, Somalia, Djibouti and Eritrea. It encompasses about 1.1 million Km² area of land and is characterized by diverse climate and topography (4). According to the recent census, the total population of the country is 74 million with an average annual growth rate of 2.6% (40). The male to female ratio is 1.02, 45% of the population is under the age of 15 years and 84% of the population dwells in rural areas. Oromo and Amhara are the major ethnic groups constituting about 61% of the population. The commonest religion is Orthodox Christian followed by Muslim and Protestants (40). The national economy is dependent on subsistent agriculture which accounts for 45% of Gross Domestic Product (GDP), and 85% of the total employment. The country is one of the least developed in the world with GDP per capita of \$1,000 (41).

According to EDHS 2011, the prevalences of stunting, underweight and wasting among under five children in Ethiopia are critically high (44.4%, 28.7% and 9.7% respectively). The estimated neonatal, infant and child mortality per 1000 Live Births (LB) are 37, 59 and 88, respectively. Nationally, the contraceptive prevalence coverage is 28.6% and the total fertility rate is 4.8. Regarding maternity care, only 33.9% and 10.0% of women who gave birth in the preceding five years of the survey received antenatal care (ANC) and delivery service from skilled providers (42). With reference to the 2009 estimate of WHO, the maternal mortality rate in the country was 470/100,000 LB and the life expectancy at birth was 54 years (43).

SNNPR, one of the nine regional states of Ethiopia, is located in the Southern and Southwestern part of the country (44). The region has an estimated population of 15 million of which 90% are rural inhabitants. With an estimated area of 112,343 km², it has density of 134 population/km² (40). SNNPR comprises 56 ethnic groups with their own distinct social identities (44). The predominant ethnic groups are Sidama, Guragie, Welaita, Hadiya and Gamo. About 56% of inhabitants are followers of Protestant Christianity. Other familiar religious affiliations are Orthodox Christianity and Islam (40). According to EDHS 2005, coverages of family planning, ANC and delivery services in the region were 11.9%, 30.3% and 3.7%, respectively (4).

Sidama Zone is one of the 15 zones and special woredas of SNNPR (40). It is bordered by Oromia region, Gedeo zone and Welaita zone. The zone is divided into 10 administrative woredas. Of the population nearing 3 million, 5.7% dwells in urban areas. With an estimated area of 6,833 km², it has density of 430 population/km² (40). According to the 1994 census, the four largest ethnic groups were Sidama (88.6%), Amhara (4.2%), Oromo (3.0 %) and Welaita (1.8%) and religion-wise about 62.5% of the population were Protestants (45). The district is characterized by three agro-ecological zones; the lowlands (20%), the midlands (48%) and the highlands (32%). Major crops grown include *Enset* (*Enset ventricosum*), Coffee and Maize. In the district population pressure, land shortage and soil erosion are the major challenges of food security (46). According to the 2004 World Bank report, in average a rural household has 4.9 dwellers, 0.3 hectares of land and 0.5 heads of livestock. About 85% of the population livelihood depends on farming and 72% of the land is exposed to malaria (47).

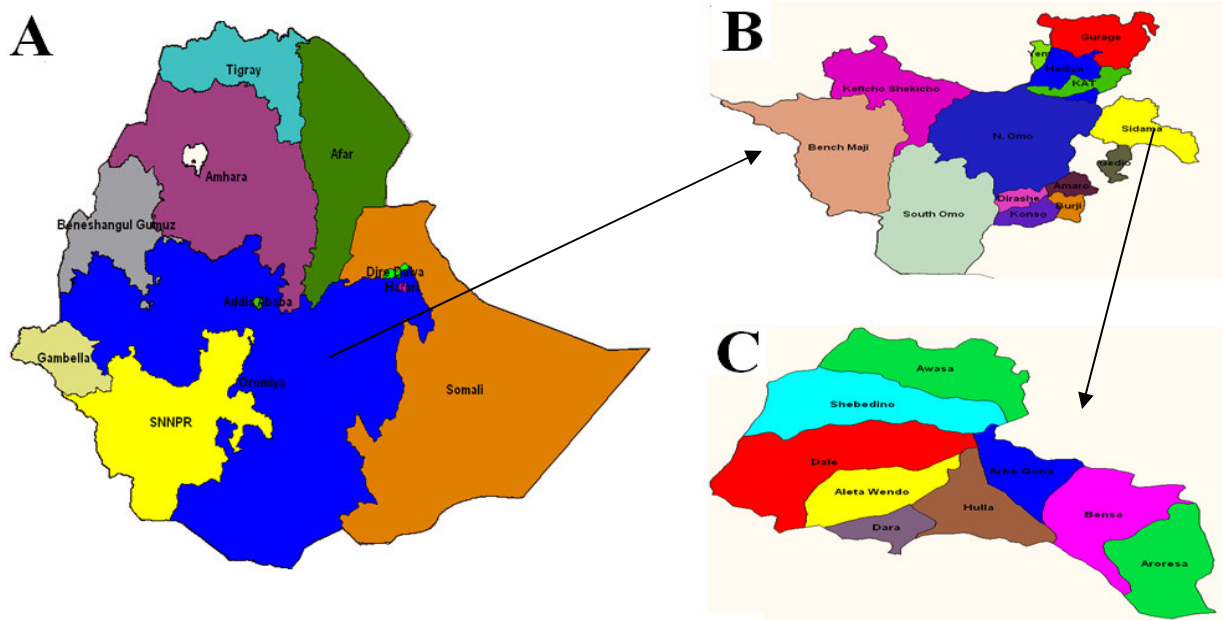


Figure 1: (A) Map of Ethiopia showing regional states and city administrations; (B) Map of SNNPR showing zones and special woredas; (C) Map of Sidama zone showing woredas.

Rationale and Significance of the Dissertation

Globally the public health significance of ZD is acknowledged recently (48). Especially in Ethiopia, scientific evidences are inadequate to initiate focused and comprehensive interventions against ZD. For instance the 2004 national guideline for control and prevention of micronutrient deficiencies did not consider ZD as a precedence intervention area (49). The 2008 National Nutrition Strategy (NNS) considered it as a priority problem but only related to its importance in decreasing the severity and duration of diarrhea in children (50). Accordingly, the current study has shaded light on the existing burden of prenatal ZD and can potentially provide input for policy makers.

Pertaining to maternal VAD, most of the earlier studies in Ethiopia were principally reliant on clinical deficiency indicators like night-blindness which symbolize merely a fraction of the underlying problem. The studies were also focused on the general group of women of reproductive age. Accordingly, the full extent of the problem in pregnant women who take the upmost burden of the deficiency has not been adequately explored. Hence the current study helps to illustrate the full scale of the deficiency in the locality.

In addition to VAD and ZD, the thesis has also presented the prevalences of Iron Deficiency (ID) and Severe Acute Malnutrition (SAM). The fact that multiple deficiencies were measured simultaneously in similar group of subjects has created the opportunity to evaluate the burden and interface of multiple deficiencies among pregnant women and the potential synergetic effects of the deficiencies in predisposing to LBW. Previous studies in the developing world did not address this aspect adequately (51).

The study explored the significance of diverse risk factors of the aforementioned deficiencies. Consequently it can identify pertinent risk factors of programmatic and scientific interest. The study may help to answer relevant questions like; does prenatal iron supplementation exert deleterious effect on maternal zinc status? Are there differences in the agro-ecological distribution of the deficiencies? How much do too close and too many births affect maternal micronutrient status? Which specific maternal age category is at higher risk of VA and zinc deficiencies? How beneficial is ANC in reducing the burden of the deficiencies? etc...

Pertaining to birthweight, most of the existing studies in the country were health institution based. Accordingly the studies may have over or underestimated the prevalence of LBW. Underestimation may have happened due to the relatively better access of the privileged urban community to the health institutions; whereas, overestimation may occur as complicated pregnancies are likely to end up in institutional deliveries. As the current study was community based, it provides additional scientific evidence on the prevalence of LBW from a relatively different perspective of view.

As birthweight is the most crucial determinant of childhood survival and health (34), from policy and programmatic perspective of views, studies evaluating the determinants of LBW are extremely valuable. Currently there is inadequate evidence on the importance of maternal VA and zinc status in causing LBW. In this regard the study has generated dependable evidences. Further, the thesis has incorporated a meta-analysis on the effect of prenatal zinc supplementation on birthweight. The analysis can help to clarify the divergence and ambiguity which exists in the literatures on the aforementioned scientific inquiry.

In general the output of this study can have significant contribution to public health and nutrition policy makers and program designers and it is of scientific interest too. Though the study was carried out in a district with its own peculiar attributes, the findings can be reasonably be informative about the existing nutritional situation in other settings of the country with similar socio-demographic and dietary characteristics.

Literature Review

Zinc Deficiency

Background Information about Zinc as a Nutrient

Zinc is one of the essential trace elements and major micronutrients which attained prominence in human health and nutrition (52). Zinc is present in all human body tissues and fluids. The total body zinc content in an adult male of 70 kg is 1.5-2.5 g. Over 80% of the total is located in skeletal muscles and bones (51).

Zinc is required for many physiological functions including DNA synthesis, cell division and gene expression. It is vital for the function of more than 300 enzymes involved in the metabolism of macronutrients, micronutrients and nucleic acids (48, 53). It involves in the stabilization of cellular structures and maintains cellular, tissue and organ integrity. Its involvement in such fundamental activities makes it indispensable for all forms of life (48).

It had been known for more than ten decades that zinc is essential for the growth of microorganisms, plants and animals. However, until 1963 it was believed that ZD could never occur in humans. Its importance for human health was first documented in 1963 when ZD was identified as a major etiological factor in the syndrome of "Adolescent nutritional dwarfism" in Egypt and Iran (48, 52-54). A decade later, severe ZD was identified among patients of Acrodermatitis enteropathica and people exclusively dependent on intravenous feeding. In 1970s and 80s, RCTs identified ZD as a cause of growth retardation in children. Studies throughout 1990s and beyond contributed to the documentation of the etiological role of zinc in several clinical circumstances including neuropsychological functions and immune impairment (52, 54).

In humans ZD is called hypozincemia. Signs of severe deficiency include low level of zinc in blood circulation, hair loss, skin lesions, diarrhea, acne, wasting and white patches on fingernails. Eyesight, taste, smell and memory are also related to zinc (54). Yet, none of the manifestations are specific to ZD. The major consequences of ZD include growth retardation, male hypogonadism, neuro-sensory changes, delayed wound healing, compromised immunity and impaired cognition (55).

A meta-analysis showed that zinc supplementation has a highly significant effect on linear growth and weight gain of children. RCTs documented that the incidence and duration of diarrhea, acute lower respiratory tract infections, malaria and skin infections decreased significantly following zinc supplementation. Studies from India, Guatemala and China illustrated that cognitive impairment could be corrected by zinc supplementation in children with ZD. Thus three major health problems in developing countries, namely growth retardation, susceptibility to infections and cognitive impairment might be related to ZD (54, 55).

In 2002 WHO ranked ZD as the fifth leading risk factor of public health, next to underweight, unsafe sex, unsafe water and indoor air pollution, in the developing world. According to the report, 3.2% of total Disability Adjusted Life Years (DALY) loss in developing countries is attributable to ZD hence it's more imperative than VAD and Iron Deficiency Anemia (IDA) (56).

Red meat, whole-grain cereals, legumes, pulses and milk provide the highest concentrations of zinc (25-50 mg/kg). Processed cereals, polished rice, and meat with high fat content have moderate content (10-25 mg/kg). Fish, roots, tubers, green leafy vegetables and fruits are only modest sources (<10 mg/kg). Separated fats and oils and sugar have very low zinc content. The bioavailability of zinc in plant source diets is limited (57).

The RDA of zinc depends on age, gender, and pregnancy and breastfeeding statuses. In both sexes the requirement is higher during puberty. Non-pregnant and non-lactating women above 18 years require 8 mg/day. Pregnant and lactating women require 3 and 4 mg more zinc daily, respectively. RDA for men is higher by a third than women (57).

Zinc absorption is concentration dependent and occurs throughout the small intestine. Theoretically zinc can be absorbed with 60-70% efficiency. However, absorption from solid diets is less efficient (20-40%). Absorption varies depending on zinc content and diet composition. Soluble organic substances such as aminoacids facilitate absorption whereas compounds like phytate impair absorption. Competitive interactions with other ions of similar physicochemical properties like iron and copper can negatively affect the uptake (58).

So far labile zinc storage sites have not been identified in human body and its homeostasis is poorly understood. Yet zinc depletion studies illustrated that circulating zinc and activities of zinc-containing enzymes can be maintained within normal range over several weeks after inadequate intake. Possible explanations include reabsorption from bone and tissue, enhanced intestinal absorption and presence of exchangeable body pool of zinc (51, 58) .

Zinc is excreted mainly via intestine (0.5-3 mg/day) and to some extent through kidneys and skin (0.5-0.7 mg/day). The level of excretion is dependent on dietary intake. Starvation and muscle catabolism increase zinc loss in urine. Strenuous exercise and elevated ambient temperatures lead to losses via perspiration. In men loss in semen can also be substantial (51, 58).

Prevalence of Prenatal Zinc Deficiency

Research in the last two decades suggested that ZD is a widespread public health problem worldwide. According to WHO, one-third of the world population is at risk of ZD and the risk ranges from 4-73% across various geographical regions. The prevalence is low (4-7%) in North America and Europe, and high in South and Central America (68%), Sub-Saharan Africa (37–62%), North Africa and Eastern Mediterranean (25–52%), and South and Southeast Asia (34–73%) (7).

The United Nations Food and Agriculture Organization (FAO) estimated the adequacy of dietary zinc intake based on the international food balance sheet. According to it, approximately half of the world's population is at risk of low intakes. Nearly the entire population in South Asia (95%), the majority in Southeast Asia (71%), Africa (68-74%) and Latin America (46%) were at risk of low intake (59).

ZD is exceedingly common in the developing world where nearly two billion people are affected (60). Few studies even reported prevalence levels exceeding the general estimates of WHO and FAO. A study in Iraq among healthy subjects found 58.4% prevalence of ZD and 74.8% of inadequate dietary intake (61). A study in Nepal among women of reproductive age documented 75% prevalence of ZD (62).

Several studies illustrated extremely high prevalence of ZD amongst pregnant women. Based on estimated zinc intakes, Caulfield et al concluded that worldwide 82% of the pregnant women are likely to have inadequate zinc intake (7). Studies based biochemical indices also confirm high burden though their cutoff points for defining ZD were highly assorted. Studies in India (63), Peru (64), Iran (65), and Indonesia (66) reported 65%, 60%, 49%, and 22% prevalences, respectively. Substantial burden has also been reported in developed countries like Sweden (18%) (67).

Very little is known about the prevalence of ZD in Africa. The only existing country specific information is the estimate IZiNCG (60). According to the estimate, all African countries are either in high or intermediate risk category for ZD. Most of South and Central Africa countries, with the exception of South Africa, Namibia and Botswana, are in high risk category. Nearly all Western, Northern and Northeastern African countries, with the exception of Eritria, are in intermediate risk category. Ethiopia was also categorized in the intermediate risk group (60).

According to a study in rural Malawi, among pregnant women at 24th gestational week, 36% had low plasma zinc level and 46% had low hair zinc concentration. Median daily intake of zinc was low and poorly available as only 20% of it was contributed by animal sources (68). A study in Southeastern Nigeria reported 45.8% prevalence of the deficiency among apparently healthy pregnant women attending Antenatal Care (ANC) (69).

In Ethiopia information on the prevalence of ZD is limited. A study conducted in rural Sidma among pregnant women in the third trimester reported high prevalences of inadequate dietary zinc intake (>99%) and low plasma zinc level (72%) (6). A study in the outskirts of Awassa, concluded that women in the third trimester were mainly dependent on high phytate diet, had low zinc absorption (6.0 ± 3.2 mg/d) and low serum zinc level (44.1 ± 6.0 μ g/dl) (70). However a study in Addis Ababa among anemic lactating women came across with merely 11.3% prevalence of marginal ZD (8).

Measuring the Public Health Significance of Zinc Deficiency

In 1997 WHO developed a criteria for measuring the public health significance of ZD. The threshold divides the risk into low, moderate or high based on the percentage of population at risk of inadequate bioavailable zinc intake and prevalence of stunting among under five children. The risk is considered as low when the prevalences of inadequate bioavailable zinc intake and stunting are less than 15% and 10%, respectively. The risk is of moderate degree when either of the aforementioned indicators attains 15-25% and 10-20% prevalence, respectively. When the indicators exceed 25% and 20% respectively, then the risk is of high degree (60).

Alternatively, according to the joint recommendation of WHO, UNICEF and IZiNCG, the risk of ZD is considered to be of public health concern when the prevalence of low serum zinc concentration exceeds 20% (71).

Correlates of Zinc Deficiency during Pregnancy

ZD is largely related to inadequate dietary intake or absorption though excess losses through diarrhea, especially among children, may contribute to the deficiency. Consumption of high levels of absorption inhibitors can lead to ZD, even if dietary intake of zinc is optimal (72).

Phytate, the principal storage form of phosphorus in many plant tissues, is the most frequently documented inhibitor of zinc absorption. Frequent consumption of phytate rich foods like soybean, maize, barley, peas and wheat can notably compromise zinc status (73). Beyond animal experiments, a study in Malawi witnessed that individuals consuming diets with higher phytate-zinc ratio had significantly lower hair zinc concentration (68). A study in Nepal also showed a negative association between plasma zinc level and phytate intake (62).

Few studies attempted to identify potential correlates of prenatal ZD. The frequently cited factors are socio-demographic and economic variables, gestational age, parity and macro- and micro-nutritional indices. Nonetheless, as the studies are few, incomprehensive and contradicting, they are not conclusive.

Maternal age is considered as a correlate of zinc status. The existing understanding is that serum zinc reaches peak during adolescence and young adulthood and then declines (51). And yet studies reported mixed findings. In Iran elevated prevalence of ZD was reported in women aged 20-30 years than younger and older age groups (65). Likewise, a study in Nigeria concluded that the prevalence of ZD increased with age, attained peak in 25-29 years age and declined in older age groups (69). In a study in Iran, no distinct pattern of association was illustrated (74).

Studies have recognized the affirmative influence of enhanced socio-economic status on zinc. A study in Nigeria found superior zinc status in socially advantaged pregnant women as measured by occupation and education (69). In Indonesia household income was a substantial determinant of zinc status (66). In India zinc status indicators were negatively associated with family size and positively related with improved environmental condition as evaluated by type of housing, ventilation, cleanliness, overcrowding and water supply (75). But studies in Iran (65) and USA (76) found no association between socio-economic status indicators and zinc status.

Gestational age is frequently cited as a predictor of zinc status. Pregnancy is associated with a decline in zinc level perhaps as early as the first trimester; however, the reduction in the later two trimesters is prominent due to hemodilution (51). A longitudinal study in Turkey witnessed a significant decline in plasma and hair zinc concentrations in the second trimester compared to the first trimester (77). But a study in Iran found the opposite (65). According to the study, there was direct association between gestational duration and serum zinc level. The prevalence of ZD was 61, 57 and 41% in the first, second and third trimesters, respectively. Another study in Iran did not find any significant association (74).

Previous studies hypothesized that iron may hamper the absorption of zinc as the two nutrients have similar physicochemical properties (78). High concentration of iron given in water found to inhibit zinc absorption and the vice versa (78). Many community based studies attempted to assess the relation between prenatal iron supplementation and zinc status. Salimi et al, reported mothers who were supplemented for iron were more likely to develop ZD (65). Similarly, Vaishali et al found that among healthy Indians zinc status indicators were negatively associated with iron intake (75). Nevertheless, in studies conducted in Nigeria (79), Iran (74, 80) and UK (81, 82), daily iron supplementation was not associated to maternal zinc status.

Several studies conducted among pregnant women reported positive association between zinc and hemoglobin levels. According to a study in Southern Ethiopia, among several covariates, zinc was the strongest predictor of prenatal hemoglobin level (18). Studies in Sudan (83), Iran (74) and Nepal (62) also found significant positive correlation between the two parameters. Nevertheless, as the studies are cross-sectional it is not viable to implicate causal relation.

The association between parity and ZD was studied in rural Malawi in which frequent reproductive cycling was related to increased risk of ZD. Multigravida women were 1.25 times more likely to have ZD (68). A study amongst low income pregnant women in USA reported lower mean plasma zinc in multiparous compared to primiparous women (76). Likewise, in Nigeria plasma zinc level was inversely related to parity (69). However, two studies in Iran (65, 74) and a study in South Africa (84) did not witness significant association.

Few studies assessed the relation between zinc and anthropometric measurements. A longitudinal study in Turkey reported better zinc status in well-nourished pregnant women (77). A study in Iran document reduced risk of ZD in women with body weights of 50-60 kg compared to women weighing less than 50 kg (85). However, in USA amongst low income pregnant women, mean plasma zinc level was lower in women with body weight greater than 70 kg compared to their counterparts (76). A study in Iran found no association between zinc and BMI (74). The frequently observed positive relationship between zinc and anthropometric indicators can be due to the clustering nature of malnutrition.

A systemic analysis demonstrated that maternal stress, alcohol abuse and cigarette smoking during pregnancy increase risk of ZD. However, the exact patho-physiological mechanisms have not been lucidly stated (36).

Serum zinc concentration is independently affected by acute inflammation/infection, diurnal variation and fasting. The concentration is reduced during acute infection/inflammation probably due to the redistribution of zinc from the plasma to the tissue. The concentration also shows diurnal fluctuation principally due to the effect of food ingestion. Following a meal, there is an immediate initial increase followed by a gradual decline. During an overnight fasting, the concentration steadily increases to reach the highest level in the morning (60, 86).

Zinc Deficiency and Birth Outcomes

Poor maternal zinc status is associated with adverse pregnancy outcomes as zinc is essential for the normal fetal growth and development. Animal experiments also indicated that maternal ZD upsets both the sequencing and efficiency of parturition (20). Difficult and prolonged labor, hemorrhage, uterine dystocia and abruption have been documented in zinc deficient animal models (20). The poor pregnancy outcomes in women with Acrodermatitis enteropathica are also consistent with effects observed in zinc deficient animals (36).

Studies in humans also linked maternal ZD with wide range consequences including spontaneous abortion, PIH, PROM, abruption, prolonged labor, hemorrhage, infections, IUGR, LBW, congenital anomalies, neonatal morbidity and poor neurobehavioral development (20, 87). However, most of the aforementioned consequences are still equivocal.

Studies attempted to evaluate the effect of prenatal ZD on maternal health and pregnancy outcome in apparently healthy individuals yielded conflicting conclusions (20, 88). Among the available 20 or so RCTs, most assessed birthweight, other anthropometric measures of the newborn and duration of pregnancy as outcome variables. Few assessed APGAR score, PIH, bleeding, and other complications. However, only a handful of the RCTs were conducted in the developing world, and the trials differed in dose and duration of zinc supplementation (36).

Among the RCTs, few illustrated the positive effect of zinc supplementation on anthropometric indicators. In India, the weight of infants born to women in the placebo group averaged only 2.6 kg. Depending on the duration of supplementation, infants born to the intervention group were heavier by 0.3-0.8 kg. Pronounced effect was observed when the supplementation was initiated earlier (89). Goldenberg et al, among disadvantaged African-American women, concluded that zinc supplementation increased birthweight by 126 g and increased infant head circumference by 0.4 cm (90). Tamura et al in USA (91) and Duran et al (92) in Chile reported about 150 and 69 g increment in birthweight after zinc supplementation. A study in Iran found considerable improvement in head circumference but not in birthweight (93). The remaining RCTs (94-105) failed to witness the positive effect of zinc supplementation on anthropometric indicators.

So far three RCTs showed significant reduction in preterm deliveries due to zinc supplementation (106). In India a study demonstrated that the gestational duration in the control group was significantly lower (38.3 weeks) compared to women supplemented with zinc starting from first trimester (39.4 weeks) and third trimester (38.8 weeks) (89). A study in US concluded the same (107). A study among economically disadvantaged Chilean adolescents found 50% reduction in prematurity due to the supplementation (92). But RCTs in UK (94), Bangladesh (95), Iran (108), USA (96), Peru (98) and Denmark (109) reported no significant association. Overall, a meta-analysis concluded that that zinc supplementation results in a small but significant reduction (about 14%) in preterm birth (35).

Regarding other pregnancy outcomes, Mohamed et al in UK investigated the possible association of zinc supplementation with maternal bleeding, PIH, APGAR score and neonatal abnormalities. However, no significant association was observed (94). A study in Iran witnessed no significant differences in APGAR score and IUGR in intervention and control groups (93). Johnson et al in Denmark found no effect on the incidence of PROM, prolonged labor, PIH and hemorrhage (109). However, Hunt et al documented reduced incidence of PIH due to zinc supplementation among adult women (96), but not in adolescents (102).

Observational studies frequently reported positive association between birthweight and maternal zinc status. A case-control study in Tanzania witnessed 2.6 times elevated risk of LBW in mothers with low zinc levels (110). Whereas, in Nigeria the mean plasma zinc level was significantly lower in LBW infants than their controls (111). According to a study in Egypt, plasma zinc level and maternal weight explained 39% of the variability of birthweight (112). In Japan serum zinc in the second trimester was not related to the birthweight but ZD in the last trimester was significantly associated to LBW (113). In India the mean birthweight of infants born to zinc deficient mothers were significantly lower by about 350 g than their counterparts (114). According to a cohort study in US, women who had serum zinc concentrations in the lowest quartile had significantly higher risk of LBW than those in the remaining three quartiles (115). Studies in Nigeria (116), India (117), and China (118) also reported positive correlation.

Assessment of Zinc Status

Since the importance of zinc for human health was recognized, there has been extensive effort to identify and validate indices of zinc status. However there has been limited success (119). So far there is lack of single, specific and sensitive biochemical index that reflects the entire spectrum of zinc status from deficiency to toxicity. However, available indices include serum, erythrocyte, leukocyte, urinary and hair zinc concentrations (51).

Serum zinc is the most commonly used biomarker. Several studies have confirmed its usefulness for assessing zinc status at population level. However, at individual level it may not be able to detect marginal deficiency as serum zinc is homeostatically controlled. Erythrocyte zinc is an option but it does not reflect short term changes in zinc status. Leukocyte zinc is more sensitive than erythrocyte zinc; however, its interpretive criterion is yet to be established. Urinary zinc is not a sensitive indicator as it only changes at extreme deficiency or high dose consumption states. Hair zinc concentration indicates chronic deficiency when protein energy malnutrition is absent. However, it is not helpful in evaluating acute deficiency state (51).

Strategies for the Control of Zinc Deficiency

At times when zinc requirement cannot be met by habitual dietary intake (like in the case of pregnant women, LBW infants and children with persistent diarrhea and malnutrition), zinc supplementation can be considered as a strategy to combat ZD. Strong evidence exists that zinc supplementation improves the prognosis of diarrheal treatment and enhances physical growth in stunted and LBW babies (88, 119, 120). However its significance in preventing pregnancy complication is equivocal (35) The major challenges of zinc supplementation are uncertainty in effectiveness, bioavailability, side effects and compliance; donor dependency, and added cost and burden on the health care system (88, 119).

Zinc fortification is a cost effective and sustainable method for improving zinc status in countries where ZD is endemic. It does not require changes in the existing food beliefs and does not impose a burden on the health sector. In western countries flour, bakery goods, breakfast cereals, cereals, and infant formulas are frequently fortified with zinc. Fortification of cereals in Ghana and Mexico has also shown encouraging results. Major challenges of this strategy are lack of

industrially processed and universally consumed foods, technological and economical feasibility, lack of bioavailability and difficulty in balancing adequate intake and toxicity. In developing countries zinc fortification is restricted in addressing the nutritional need of population in refugee camps and pregnant mothers and children enrolled in supplementary feeding programs (88, 119).

Dietary modification and diversification is a more sustainable, long term, economically feasible strategy that can be used to alleviate several micronutrient deficiencies simultaneously. It involves changes in food selection patterns with the overall goal of enhancing the access, availability and utilization of foods with a high content and bioavailability of zinc throughout the year. Common interventions under this option includes promoting greater consumption of animal source foods, increasing the zinc content of staple foods by selecting and introducing zinc efficient genotypes or augmenting the use of zinc fertilizers (88, 119).

In areas where phytate is a major cause of ZD, phytate reduction via household food processing techniques like soaking, fermentation and germination of seeds can be considered. Soaking is a practical method to reduce the phytate content of certain cereals (e.g. maize), and most legumes. About 10-70% of the phytate in these staples is stored in water soluble form and hence can be removed by diffusion. Germination of seed for 2-3 days can increase the breaking down of phytate by 23-88% depending on the type of the grain. Fermentation induces phytate hydrolysis via the action of microbial phytase which can originate either from the microflora on the surface of the seeds or by inoculation with microbial starter (119, 121-123).

Vitamin A Deficiency

Background Information about Vitamin A

VA includes several bioactive compounds known as Retinoids as well as precursor forms of the vitamin known as Carotenoids. Retinoids are preformed VA obtained primarily from animal sources. Retinoids exist in different chemical forms including retinyl, retinol, retinal, and retinoic acid. Carotenoids, provitamin A, are yellow-orange pigments found only in plant sources. Varieties of Carotenoids include alpha, beta and gamma carotenes. All forms of VA have a β -ionone ring to which an isoprenoid chain is attached (124).

VA is an essential micronutrient needed for the normal functioning of the visual system, growth and development, epithelial integrity, immune function and reproduction. In the visual system, rhodopsin is formed in rod cells through the conversion of trans-retinol to cis-form, and binding to opsin. The growth and differentiation of epithelial cells are affected by VAD. During VAD, goblet cells in epithelial tissues are reduced and antimicrobial secretions diminish. Protective tissue surfaces fail to regenerate and differentiate; hence they flatten and accumulate keratin. Both changes reduce the body's ability to resist microbial invasion (125, 126). VA is also required for the development of white blood cells. The activation of T-cells requires the binding of retinoic acid with cellular receptors (127). Other functions of VA include the building of strong bone and teeth and the formation of erythrocytes and ribonucleic acid (125, 126).

Since the ancient Egyptian times it was known that certain foods, such as liver, would cure night-blindness. However, VA was not identified until the beginning of the 20th century. Though a couple of scholars in late 19th and early 20th centuries postulated the importance of “minimal qualitative factors” in diet for growth and survival of animals, a study that led to the discovery of VA was given by EV McCollum. During 1907-12, he found that young rats on pure protein, milk sugar, minerals and olive oil diet failed to grow, whereas adding butter or egg yolk restored their health. Subsequent experiments proved the importance a fat-soluble growth factor in butter. This factor was named as “fat-soluble factor A” (128, 129).

In 1930's experiments established the precursor nature of carotene, chemical structure of VA, and the RDA of the nutrient. In 1940's studies identified the importance of VA for vision, epithelial differentiation and growth. In 1950s and 60s, the metabolism of VA was delineated. In the 1970s and 80s, its anti-carcinogenic action was discovered. In 1987 two studies discovered the retinoic acid receptors in cell nuclei. The finding led to the hypothesis that VA can affect gene expression and as it has hormonal nature of external origin (128, 129).

WHO defines VAD as tissue concentrations of VA low enough to have adverse health consequences even if there is no evidence of clinical deficiency. Clinical deficiency is manifested by night-blindness, xerophthalmia and irreversible blindness due to complication of xerophthalmia. Nonspecific symptoms include morbidity, poor reproductive health, anemia, slow growth and development. However, non-ocular symptoms are not specific to VAD (125).

Preformed VA is found in animal sources like glandular meats, liver, fish, egg yolk, milk and dairy products. Provitamin VA is richly found in dark green leafy vegetables and orange, yellow and red fruits and vegetables (125, 130). The RDA for VA ranges from 300-1300 μg depending on age, sex and, pregnancy and lactation status. RDA for pre-puberty children ranges from 300-600 μg depending on age (131, 132). Non-pregnant and non-lactating women aged 14 years or above require 700 μg VA, which is 200 μg less than that of men. During pregnancy and lactation the RDA elevates to 770 μg and 1300 μg , respectively (130).

VA is stored to a variable degree in the body, making it more likely to cause toxicity when taken in excess. Presentation of toxicity includes nausea, jaundice, irritability, anorexia, vomiting, blurry vision, headaches, hairloss, muscle and abdominal pain and weakness. Acute toxicity occurs at doses of 25,000 IU/kg of body weight, while chronic toxicity occurs at 4,000 IU/kg of body weight daily for 6-15 months. Toxicity is only associated with preformed VA (133).

VA is absorbed in the small intestine with 70-90% efficiency. The efficiency continues to be high (60-80%) as intake increases (110). The rate of absorption is higher for proformed VA. The main dietary sources of VA are enzymatically converted to retinol in intestinal lumen (132).

In addition to the storage form called retinyl ester, in human body VA has three active forms namely retinal, retinol and retinoic acid. Bilateral conversion is possible between retinyl ester and retinol, retinol and retinal while retinal can be changed unidirectionally to retinoic acid. Circulating retinol is primarily bound to Retinol Binding Protein (RBP), and can enter and leave the liver. Other forms are transported via low density lipoproteins. The three active forms serve different but overlapping functions. For instance retinal is required for rhodopsin formation and vision, while retinoic acid is needed for epithelial growth and differentiation (134).

Prevalence of Vitamin A Deficiency

Preschool-age children and pregnant women are population most at-risk for VAD due to their increased demands for VA and the potential consequences associated with VAD. Out of the 193 WHO member states, VAD, as indicated by night-blindness or biochemical deficiency, is present in moderate to severe degrees in preschool-age children in 45 and 122 countries, respectively. VAD in pregnant women is less prevalent than in children; and yet, either night-blindness or sub-clinical VAD is present as moderate to severe problem in 66 and 88 countries, respectively (16).

In 1995 WHO estimated the global distribution of VAD. Accordingly, about 3 million children had some form of xerophthalmia and another 250 million had subclinical deficiency. The prevalence was highest in Southeast Asia (69%), followed by Africa (49%), West Pacific (27%), Eastern Mediterranean (22%) and the Americas (20%) (16). According to the 2002 estimate of International Vitamin A Consultative Group (IVACG), globally 127 million preschool-aged children had VAD of which 44% live in South and Southeast Asia and, 26% and 10% live in Africa and Eastern Mediterranean region. The largest numbers of deficient children live in India (35.3 million), Indonesia (12.6 million), China (11.4 million), and Ethiopia (6.7 million) (135).

According to WHO, globally night-blindness affects 9.8 million pregnant women (95% CI: 8.7-10.8 million), which corresponds to 7.8% (95% CI: 7.00-8.70%) of the population at risk. Low serum retinol ($<0.70 \mu\text{mol/l}$) affects 19.1 million pregnant women (95% CI: 9.30–29.0 million) which corresponds to 15.3% (95% CI: 7.40-23.20%) global prevalence. Region specific estimation indicates that the prevalence of subclinical deficiency is highest in West Pacific (21.5%) and Southeast Asia (17.3%) followed by Eastern Mediterranean (16.1%) and Africa (13.5%) (16).

Several studies over the years have established the public health significance of VAD in Ethiopia. Studies since late 1950 have consistently indicated that the prevalence of Bitot's spot among preschool-age children is well above the cutoff point of 0.5% which is indicative of its public health significance. Recent studies reported the same (136-139).

According to WHO, between 1995 and 2005, among Sub-Saharan Africa countries Ethiopia had the highest burden of VAD in terms of prevalence of maternal night-blindness. It was estimated that 13.2% (95% CI: 1.5-59.6%) of all pregnant women in the country had low serum retinol level. The prevalence was equivalent to 424,000 VA deficient pregnant women (16).

EDHS 2005 testified that 6.1% of pregnant women in the country had reported night-blindness during their recent pregnancy (4). The highest burden was reported in Amhara (11.7%) followed by Tigray (8.3%) and Benishangul-Gumuz (5.8%) regions. The prevalence in SNNPR was 2.6%. The lowest prevalence was reported in Addis Ababa city (0.9%) and Harari region (1.2%) (4). Likewise, EDHS 2000 reported 4.8% national prevalence of night-blindness during pregnancy. The highest prevalence (16.5%) was reported in Tigray region (140).

Based on biochemical markers, three studies in Ethiopia witnessed the public health significance of VAD in pregnant women. A study in rural Sidama reported 0.96 (± 0.35) and 0.76 (± 0.35) $\mu\text{mol/l}$ serum retinol among women from maize and *enset* (*Enset ventricosum*) staple diet areas (18). The overall prevalence of VAD was 27% (18). A study amongst ANC attendants in Gondar hospital found 1.23 (± 0.5) $\mu\text{mol/l}$ mean serum retinol concentration. The level was under 1.05 and 0.7 $\mu\text{mol/l}$ in 38.5 and 17.2% of the subjects, respectively (17). Another study in Gondar hospital documented 0.96 (± 0.42) and 1.10 (± 0.45) $\mu\text{mol/l}$ serum retinol level and, 18.4 and 17.7% VAD in HIV infected and uninfected women, respectively (19).

Measuring the Public Health Significance of Vitamin A Deficiency

The thresholds of clinical deficiency (Bitot's spot or night-blindness) and low serum retinol level among preschool-age children and pregnant women are commonly used to classify the public health significance of VAD in a community (16). Prevalence of Bitot's spot in preschool children and night-blindness in pregnant women above the cutoff point of 0.5% and 5%,

respectively are indicative of the public health significance of VAD. Higher prevalence of corneal xerosis or ulceration (>0.01) and xerophthalmia related corneal scar ($>0.05\%$) also warrant public health intervention (141). Prevalence of night-blindness among children aged 24-71 months can also be used for describing the degree of severity of VAD. Prevalence between 0-1% indicates mild, prevalence between 1-5% and greater than 5% indicate moderate and severe VAD. Prevalence of low retinol level in preschool-age children or pregnant women between 2-10%, 10-20%, $>20\%$ indicate mild, moderate and severe VAD, respectively (16).

Correlates of Prenatal Vitamin A Deficiency

VAD occurs within an ambience of ecological, economical and social deprivation in micro and macro environment in which populations live. Poverty is the root, though not the exclusive, cause as only costly animal source foods contain the preformed VA. At macro level, hostile environment conditions like infertile land determine the variety, amount and duration of availability of VA rich foods. Social under development in the community limits accessibility to services including health and education services that indirectly affect deficiency status at individual level. As a result of such multiple distal factors VAD tend to be clustered (141).

Researchers in the developing world have also identified relevant individual and household level correlates of prenatal VAD. The frequently cited factors are maternal age, gestational duration, socio-economic variables, parity, anthropometric measurements and various clinical conditions.

According to the US national health and nutrition examination survey, serum retinol in females increases in the first two decades and remains constant between the ages of 20 to 49 years (142). Yet other studies reported mixed patterns. According to EDHS 2005, the prevalence of night-blindness steadily increased from 4.3% in teenagers to 7.3% in women aged 30-34 years (4). In Nepal higher risk of night-blindness was observed in teenagers and women aged over 30 years (143). In Southern India, lower risk was documented in teenagers compared to women aged 35-49 years (144). Studies in Nepal (145), India (24) and Brazil (146) did not observe any significant association between maternal age and VA status.

Pertaining to gestational duration, several studies showed inverse association with VA status. Among Nigerians a steady decline of serum retinol level from 37.8 µg/l in the first to 21.5 µg/l in third trimester was observed (147). In Zimbabwe the level declined from 1.03 µmol/l in women less than 25 weeks of gestation to 0.82 µmol/l in women greater than 32 weeks of gestation (148). In India higher risk of maternal nightlines was observed in advanced gestational age (149). A study in Indonesia also reported 20% reduction in retinol from the first to third trimester (150).

Several studies reported increased risk of VAD in socially underprivileged pregnant women. According to EDHS 2005, prevalence of night-blindness was elevated by more than twofold among women from poorest households (4). The prevalence was also higher amongst illiterates and rural residents (4). A study in Gondar identified that women with low retinol were likely to live in earth-floored and mud-walled houses, and less likely to own a house and to have a latrine (17). Among Nepalese women, cases of night-blindness were more likely to be from the lower castes, be illiterate, live in poorer quality homes and own no land (28). In Bangladesh, per-capita expenditure on food and wealth index were significant predictors of serum retinol (151). However, a study in Brazil did not witness any relation between VA and income and educational status indicators (146).

Earlier studies reported the negative effect of extended parity on VA status. According to EDHS 2005, the prevalences of night-blindness in parity groups of 1, 1-5 and greater than 5 were 4.8, 6.1 and 6.8, respectively (4). In Southern India two times increased risk of maternal night-blindness in grand multiparas than nullparas was documented (144). Another study in India witnessed increased risk of VAD in women with advanced parity and shorter birth intervals (149). Studies in Cambodia (152) and Nepal (143) found 1.5 and 1.6 times higher risk of night-blindness in women with 3 or more previous births. However, according to a study in Brazil, the risk wasn't associated with parity and inter-gestational interval (146). Studies in Nepal (28) and India (24) also reported null association.

Studies linked inferior anthropometric status with higher risk of VAD. In Bangladesh, maternal MUAC showed a positive association with serum retinol (151). In Nepal, women with night-blindness were more undernourished than controls as indicated by weight (145). In Cambodia maternal BMI less than 18.5 was association with nearly two fold increased risk of night-blindness (152). The observed relation between VAD and anthropometric status can be due to the clustering nature of malnutrition. In addition, protein deficiency can compromise serum retinol as production of RBP requires protein substrate. Consumption of low fat diet and low fat store can also affect VA status since absorption and transportation of VA is fat dependent (51).

Studies in India and Brazil documented positive association between ANC and VA status. In Brazil, women who had five or fewer antenatal check-ups had a greater prevalence (26.7%) of gestational night-blindness than those who had 6 or more visits (15.4%) (146). In India, relatively higher, but statistically insignificant, trend of maternal VA concentrations and statistically higher values of cord VA levels were seen in mothers who received ANC (24).

Studies have identified various illnesses as risk factors of VAD. In Northwestern Ethiopia substantially lower serum VA was documented in HIV positive pregnant women (19). In Zimbabwe, serum β -carotene and retinol were lower in HIV infected women than in uninfected women. Women with malaria also had inferior VA status (148). Among Cambodian women diarrhea was associated with twofold increased risk of night-blindness (152). In children HIV, parasitic infections and measles are known to compromise VA status (51). As VA is a vital input for immunity, infection may cause deficiency through escalating biological demand. However, inverse causation and hyporetinemia due to acute phase response may not be excluded.

Intuitively risk of VAD is inversely related to consumption of VA rich foods. Studies have also availed empirical evidences. In Nigerian pregnant women, a significant correlation was observed between 24 hours VA intake and serum retinol concentration. Further women who acquired most of their VA from plant sources had lower serum retinol than those dependent on preformed sources (147). In Nepal maternal night-blindness was associated with less frequent consumption of preformed VA and provitamin A foods (28). Parallel finding was also reported in India (149).

Effect of Vitamin A Deficiency on Birth Outcomes

Supposedly prenatal VAD is related with various pregnancy complications. To date studies have linked it with maternal anemia (21, 22), prematurity (22-25), IUGR (24, 26), malformations (27) infections (28), PIH (28-31), vertical transmission of HIV (32), poor infant growth (21), infant mortality (21, 33) and maternal mortality (33). However, existing evidences are inconclusive.

An RCT in Nepal evaluated the effect of weekly low-dose perinatal VA and β -carotene supplementation on maternal mortality. As compared to the control group, VA and β -carotene supplementation yielded 40% and 49% reductions of maternal mortality, respectively. In combination, either of the supplementation lowered maternal mortality by 44% (33). However, similar RCTs in Ghana (153) and Bangladesh (154) did not witness parallel findings.

VA is believed to have importance for fetal growth (155), however, its effect on birthweight is controversial. Few studies attempted to evaluate the effect of vitamin A Supplementation (VAS) on birthweight among HIV positive pregnant women. A study in Malawi illustrated that babies born to HIV positive women who received VAS during pregnancy had substantially higher birthweight than controls (21). The proportion of LBW was also significantly reduced (21). However, an RCT in Tanzania did not witness beneficial effect of VAS in terms of birthweight or prematurity (156). In India, dietary supplementation trial during pregnancy with VA rich palm oil failed to improve birthweight and gestational duration (157).

Observational studies on the relationship between maternal VA status and birthweight concluded divergently. A study in Israel reported that cord retinol and gestational duration significantly explained more than a quarter of the variability in birthweight (37). Low retinol was also more common in infants weighing less than 3 kg (37). Studies in India (24), Guatemala (26) and UK (25) similarly found positive correlation between cord retinol and birthweight. The later study also reported positive association with birth length and head circumference (25). A study in India documented that night-blindness was associated with an increased risk of LBW (158). The lower the cutoff point used for defining LBW, the stronger the association with night-blindness (158). In USA, among HIV positives, 4.6 and 7.0 times increased risks of LBW were reported in women who had low and very low VA levels prior to the third trimester (159). However studies in Bangladesh (38) and USA (39), reported no significant association.

A trial in South Africa showed that HIV positive women receiving VAS were less likely to have preterm delivery and the prevalence of prematurity was 11.4% in the VA and 17.4% in the placebo groups, respectively (160). An observational investigation in UK (39) and India (24) also reported parallel findings. However, the association between VA and prematurity has not been replicated in other studies (161). RCTs conducted in Tanzania (32) and Malawi (21), concluded that VAS has no effect on prematurity among HIV positive women.

There is also a scientific understanding that perinatal VAD might be associated with increased risk of maternal to child transmission of HIV infection. However, the available few RCTs concluded inconsistently. Trials in South Africa (160) and Malawi (21) reported no association, but the trial in Tanzania reported 35% reduction in transmission due to VAS (162).

In animal models VAD has been associated with reduced fetal survival. However, this has not been consistently supported by studies involving human subjects (161). A recent meta-analysis of four RCTs in HIV positive pregnant women reported that perinatal VAS is not associated with incidence of stillbirths (162).

Assessment of Vitamin A Status during Pregnancy

VA status can be appraised using various indices including serum retinol, serum RBP, serum retinyl ester, Relative Dose Response test (RDR), night-blindness and Conjunctival Impression Cytology (CIC) (51).

Serum retinol is the most commonly used index but it reflects the VA status only when the liver stores are severely depleted or excessively high. It is also temperature and photosensitive. Serum retinyl ester is an alternative especially for recognition of hypervitaminosis. RBP concentration is a proxy index to serum retinol. however, it lacks established interpretive criterion. The RDR test compares holo-RBP concentration before and after VAS in order to estimate liver VA store. In depletion state the rise in RBP will be small. Night-blindness is the earliest sign of VAD commonly reported among women and older children yet it only designates moderate to severe deficiency. CIC detects early pathological characters of VAD on the epithelial cells of the conjunctiva. During suboptimal conditions cells appear enlarged, separated and keratinized (51).

Strategies for the Control of Vitamin A Deficiency

VAS is a highly effective and quick means of improving VA status of children and women in the post partum period. It is especially important in countries where food fortification is not feasible. However, its importance in the reduction of childhood illness and death is still controversial. Though VAS is assumed to be a short term strategy, it has been in effect for decades in some countries. VAS is not an accepted strategy to combat VAD in pregnancy due to its adverse effects (163).

Fortification of milk products and other products consumed by infants and young children has been in place in most industrialized countries. There is now growing experience with VA fortification in developing countries. Good examples are sugar fortification in Central America and Zambia, and maize fortification in Zimbabwe. Fortification is an effective and sustainable strategy given industrially processed and universally consumed foods are available (163).

The other strategy is the use of dietary approaches. This is the most sustainable but challenging option. It includes promotion of consumption of animal source foods through various strategies including household poultry production; and promotion of home gardens, particularly those based on fruits and dark green leafy vegetables (163).

Interaction between Zinc and Vitamin A Deficiencies

Zinc status influences various aspects of VA metabolism, including its absorption, transport and utilization (164). Postulated mechanisms include the regulatory role of zinc in the synthesis of RBP, the requirement of zinc dependent enzyme for conversion of retinol to retinal and importance of zinc for secretion of bile and hence absorption of VA (164). The other way round, the potential importance of VA for the absorption of zinc has also been documented (165).

Studies in experimental animals indicated multifaceted interaction between zinc and VA (164). An experiment in pregnant rats fed various combinations of adequate, marginal and deficient zinc and retinol diets. VA status was reduced by low intake of the both nutrients and there was interaction regard to plasma VA and fetal malformation (166). In a similar experiment, VA status was affected by the amount of zinc but not VA in the diet (167). A study in pregnant monkeys also suggested the presence of an interrelationship among zinc, VA and RBP, and indicated that marginal zinc status may alter VA metabolism (168).

Cross-sectional studies that evaluated the association between the two micronutrients concluded inconsistently (164). However, co-occurrence is expected as the micronutrients have common dietary sources. In African children with measles a significant correlation between the two nutrients was observed (169). A study in Britain among children aged 4-18 years witnessed significant association only in boys (170). Whereas, studies among healthy children aged 24-72 months in Brazil (171) and 5-12 years in Bangladesh (172), did not find any association.

Most of the existing RCTs supported the existence of interaction. In Indonesia higher plasma retinol was observed in pregnant women who received zinc supplements and, infants born to β -carotene and zinc supplemented mothers had higher retinol concentrations compared to those who got only one of the supplements (173). An RCT in Mexican children documented substantial increment in retinol after zinc supplementation (174). Another trial among old and middle aged men and women reported the same (175). A study in Bangladesh documented the synergetic interaction among the two micronutrients in reducing the prevalence of persistent diarrhea and dysentery among children (176). According to a study in Nepal zinc potentiated the effect of VA in restoring night vision among night-blind pregnant women with low initial serum zinc concentrations (177).

Low Birthweight

Prevalence of LBW

LBW is defined as weight at birth of less than 2,500 g (34). The cut-off is based on epidemiological evidences that infants weighing less than 2,500 g are approximately 20 times more likely to die than normal babies (178). LBW is either the result of prematurity or IUGR (34). LBW is the single most important predictor neonatal survival and a significant determinant of post-neonatal infant mortality, childhood morbidity (34) and cognitive development (179). The fetal programming hypothesis also linked it with markedly increased risk of cardiovascular disease and diabetes in adult life (180).

Prevalence of LBW has long been used as an imperative public health indicator. At individual level it predicts child health and survival meanwhile at population level it measures a multifaceted public health problem including maternal malnutrition, ill health, workload and poor perinatal care (181, 182). According to UNICEF and WHO, globally 15.5% of all births, more than 20 million babies, are born with LBW. The prevalences in least developed, less developed and developed countries were 18.6, 16.5 and 7.0%, respectively. The burden showed significant discrepancies across various regions. The lowest figures were reported in Europe (6.4%) and North America (7.7%) whereas the highest in Asia (18.3%) and Africa (14.3%). About 72% and 22% of all LBW births happen in Asia and Africa, respectively (182).

In Ethiopia there is scarce evidence regarding to the prevalence of LBW. UNICEF estimated 20% national prevalence (183). EDHS 2000 (140) and 2005 (4) reported 8% and 14% prevalences based on mothers' subjective impression of birth size. A community-based study in Southwestern Ethiopia found 10% prevalence and mean birthweight of 3,065 g (184). Most of the remaining studies were hospital based. A retrospective study in Tikur Anbessa hospital, based on 20 years data review found 3162 g mean birthweight and 8.4% prevalence (185). A prospective study in four major hospitals in Addis Ababa documented 3,065 g birthweight and 9.1% incidence of LBW (186). Two studies in Jimma hospital (187, 188) and studies in Gondar (189) and Metu (190) hospitals reported 11.0, 22.5, and 15.4 and 8.6% prevalences, respectively.

Determinants of Low Birthweight

Kramer based on systematic review of several studies developed a framework of established risk factors of IUGR and gestational duration with their relative importance (34). Pertaining IUGR, it classified the risk factors into direct and indirect. Maternal age and socio-demographic factors were categorized as indirect factors whereas infant sex, race, maternal stature, pre-pregnancy weight, gestational weight gain, maternal birthweight, paternal size, parity, prior history of LBW, calorie intake, general morbidity, malaria and substance use were considered as direct factors. In developing countries, among the direct factors, non-white race, low calorie intake or weight gain, low pre-pregnancy weight, short stature and malaria were reported to have profound importance explaining about 75% of the total LBW incidence (34).

Studies identified race as a determinant of LBW. According to a study, over ten years the prevalence of LBW in New York City among non-blacks consistently ranged between 6-8%. However, among blacks it was around 12% (191). In another study, LBW was substantially rare in whites than blacks and there was significant variation within blacks as determined by maternal nativity (192).

Socially deprived women have high risk of LBW delivery as they are likely to face long standing mal-exposures (193). Studies have offered empirical evidences. According to a study in India, among many determinants of LBW, maternal socio-economic status had the highest attributable risk percent (193). In Iran women with primary education were nearly 8 times likely to have LBW babies as compared to those with college level of education. Further women with unskilled worker husbands had 2.5 times increased risk (194). In Bangladesh illiterate women had 8 times increased risk than those with some education. In addition housewives and mothers with low income had substantially increased burden (195). In Lithuania women with low education, low income, unemployment and unstable marital status had increased risk of LBW (196).

Primiparity and grand multiparity are associated with increased frequency of obstetric complications including placenta-previa and abruption which can predispose LBW (181). A study in Zimbabwe reported 1.4 times increase risk of LBW in primies compared to women with 2-5 parity (197). Parallel finding has been reported in India (198). A study in USA concluded that birthweight increased from parity of 1 to 3, but dropped markedly in advanced parity (199).

Studies showed that short or long inter-pregnancy interval increase risk of LBW. Short interval may increase the risk as it can result in inadequate replenishment of maternal store whereas longer intervals compromise mothers' ability to facilitate fetal growth (181). A meta-analysis reported that compared to interval of 18-23 months shorter than 6 months interval was associated with 1.6 times increased risk. Intervals of 6-17 months and longer than 59 months were also linked with the risk. The study concluded that the association between birthweight and inter-pregnancy interval can be explained by J shaped curve (200).

Pertaining to maternal age, the occurrence of LBW has been described by a U-shaped curve, with high risk at the extremes ages (181). In USA, younger teenagers found to have significantly higher risk of delivering LBW and premature babies compared to older mothers (201). Another study also reported increased rate very LBW rate amongst adolescents in USA (202). Among women aged above 35 years, high prevalence of age-dependent illnesses might have elevated the risk. A systematic review concluded that there is insufficient evidence to consider older maternal age a direct risk factor as age-dependent confounders have not been frequently controlled (203).

The positive effect of better maternal anthropometry on LBW is plausible as maternal weight, height and pre-pregnancy weight indicate cumulative maternal nutritional status (181). Studies also availed empirical evidences. A study in Central African Republic concluded that birthweight increased in average by 20 and 15 g for each kg of maternal weight and gestational weight gain (204). In India significant correlations between birthweight and maternal weight, height, MUAC, and BMI were witness (205). In Bangladesh 260 g increase in birthweight was associated with every 1 kg increase in maternal weight at ANC registration (206).

Studies linked various pregnancy complications and clinical conditions with LBW. Among pregnancy complications the effects of PIH and anemia are well established (181). Placenta previa and abruption have also been identified as risk factors (207, 208). Among medical conditions, malaria is frequently related with LBW. In Sudan 1.7 times increased risk of LBW was reported in women with prenatal history of malaria (209). Studies in Senegal (210) and Papua New Guinea (211) concluded the same. LBW has also been associated with tuberculosis (212, 213), chronic renal disease (214), asthma (215), gonorrhoea (216) and syphilis (216).

Exposure to various prenatal stressors may lead to hormonal changes that may hamper fetal growth (181). Epidemiological studies have also linked depression and stress with LBW. A study in USA reported increased risk of prematurity and LBW with maternal stress (217). A study in Pakistan found that infants of depressed mothers had lower birthweight (2910 g) than infants of non-depressed mothers (3022 g) (218). A systematic review of more than 50 studies concluded that women exposed to acute stress in early pregnancy and to distress (anxiety and depression) in late pregnancy were at significantly increased risk of premature birth (219).

Substance use during pregnancy has very strong adverse effects on pregnancy outcomes, including prematurity and IUGR (181). Epidemiological evidences showed that tobacco use during pregnancy results in approximately 70 to 250 g reduction in birthweight (181). Passive smoking also results in approximately 25 g reduction (181). The negative effects of alcohol (220) and caffeine intake (221) on birthweight have already been documented. Nevertheless, the exact biological mechanism is not clear (181).

Reasonable number studies witnessed the deleterious effect of domestic violence on birthweight. Study in Uganda (222) and USA (223) found 4 and 2 times increased risk of LBW in women exposed to domestic violence, respectively. In Brazil however a study failed to witness similar relation (224). A meta-analysis indicated that in women who face domestic violence during pregnancy, the risk of LBW is elevated by 1.5 times and the weight is significantly reduced by 20 g (181). The effects of violence can be either direct or indirect as trauma to the abdomen can initiate preterm labor and indirect influences like stress can prompt IUGR (181).

Quite a lot studies investigated the relationship between maternal exposure to air pollution and birthweight. Ambient air pollution has been identified as risk factor in studies conducted in China (225), USA (226) and Hungary (227). Then again a meta-analysis found that indoor air pollution is associated with 1.4 times increased risk LBW, and it reduces birthweight by 96 g (228). Further evidences have been provided by studies conducted in developing countries like Pakistan (229) and Zimbabwe (230).

Altitude is thought to retard fetal growth as the lower oxygen content of air at higher elevations may result in fetal hypoxia (181). An average of 102 g birthweight decline per 1000 m elevation has been witnessed in a study carried out in USA (231). A study in Bolivia identified high altitude as an important determinant of LBW irrespective of household economic status (232). A study among Mexican-Americans found a significant but gradual increment in LBW and decline in birthweight across increasing categories of altitude (233).

The effect of maternal physical exercise during pregnancy on birthweight is controversial (181). However, few studies in the developing world witnessed the deleterious effect of strenuous physical exercise during pregnancy. A study in India found that women belonging to the highest tertile of physical activity level in the first trimester had significantly increased odds of having a baby in the lowest tertile of birthweight (234). According to a longitudinal study in Thailand, the risk of small for gestational age birth was elevated for women working longer hours (234).

Reasonable number of RCTs reported the positive effect of maternal Multiple Micronutrient Supplementation (MMS) on birthweight. A trial in Nepal found a significant 77 gm birthweight difference between intervention and control groups (235). In rural Burkina Faso birthweight and length were significantly higher in the intervention group (236). In rural Niger compared to iron-folate supplemented group the birthweight was significantly increased by 67 gm in MMS group (237). A meta-analysis of 12 RCTs, reported that MMS is associated with a significant reduction in LBW (238).

Conceptual Framework

In order to assist the evaluation of correlates of prenatal VA and zinc deficiencies, a conceptual framework was constructed based on the findings of the literature review (Figure 2). The framework comprehensively explains the risk factors of maternal malnutrition including macro- and micro-nutrients deficiencies. It classifies the risk factors into three levels; root, underlying and immediate causes. It also presents the interrelationship and mediation among them.

Factors which can directly result in malnutrition are considered as immediate causes. Immediate causes include suboptimal dietary intake, maternal store depletion due to various underlying causes, inhibition of absorption secondary to anti-nutritional factors and complex interaction of nutrients, illness, increased physiological demand due to pregnancy and illness, lack of micronutrient supplementation and pregnancy complications. Underlying factors are mediators between root and immediate causes. The category includes lack of maternity care, household food insecurity, lack of nutritional awareness, food taboos, prolonged breastfeeding, type of staple diet, maternal workload and too many and too close births. Root causes are the ultimate sources of malnutrition. The category comprises factors like community level food insecurity, gender inequality, natural resource degradation, cultural norms, values and beliefs, poverty, poor access to basic infrastructures and social services.

Due to various feasibility constraints the current study did not address all of the factors represented in the framework. The investigated factors include agro-ecological zone, household economic status, maternal employment, maternal education, maternal workload, parity, birth interval, household food insecurity, diet diversity, consumption of animal source foods, type of staple diet, physical access to health care etc.

On the other hand, “Kramer’s comprehensive framework on determinants of birthweight” (34) was adopted in order to assess the effect of zinc and VA deficiencies on birthweight. The overview of the framework is discussed under the literature review section. In the current study VA and zinc deficiencies were considered as direct determinants of birthweight.

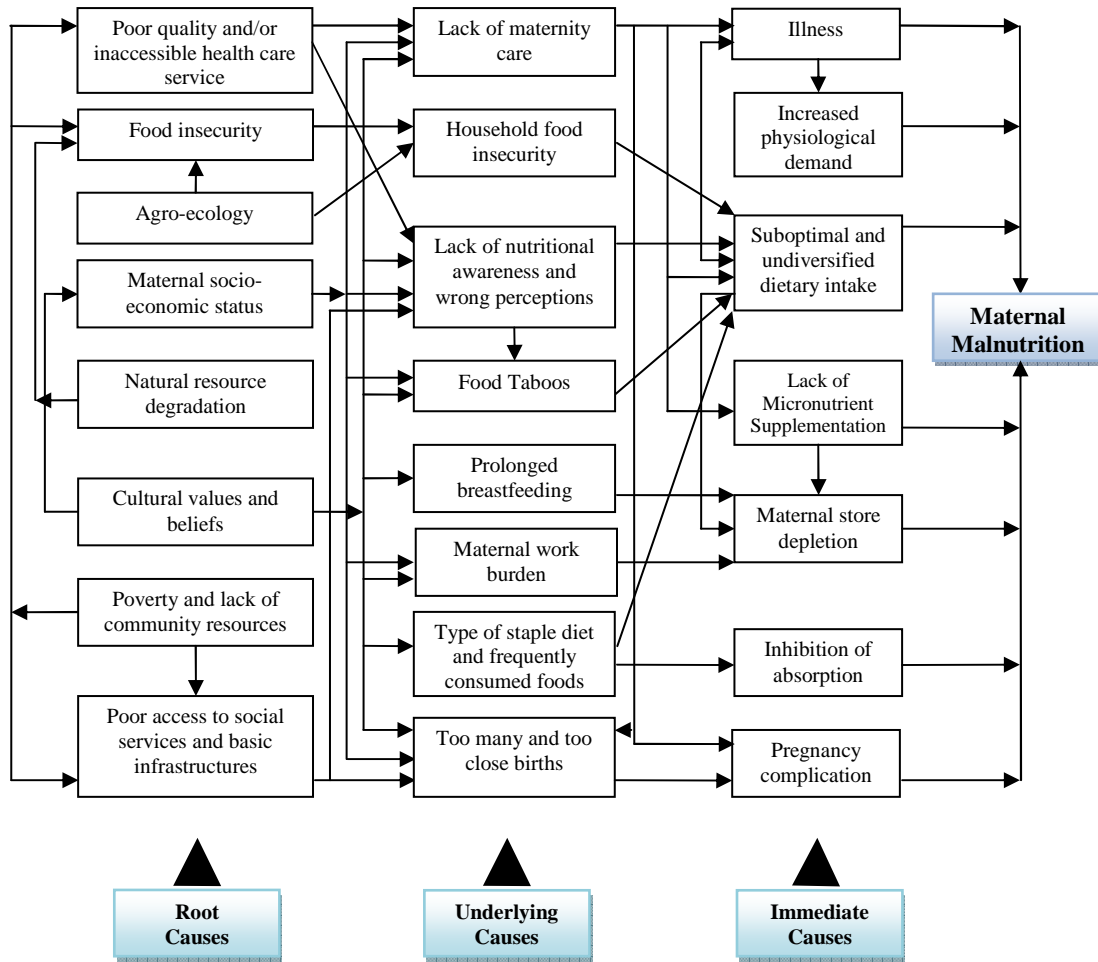


Figure 2: Conceptual framework for assessing determinants of prenatal zinc and vitamin A deficiencies

III. Objectives of the Study

General Objective:

- To determine the magnitude and factors associated with prenatal zinc and vitamin A deficiencies and their effect on birthweight in rural Sidama zone, Ethiopia.

Specific Objectives:

- To determine the prevalence of prenatal zinc deficiency in rural Sidama zone, Ethiopia.
- To determine the prevalence of prenatal vitamin A deficiency in rural Sidama zone, Ethiopia.
- To identify correlates of prenatal zinc deficiency in rural Sidama zone, Ethiopia.
- To identify correlates of prenatal vitamin A deficiency in rural Sidama zone, Ethiopia.
- To assess the effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama zone, Ethiopia.

IV. Methods and Materials

Study Design

The study applied community-based quantitative design with both descriptive and analytic elements. The study used cross-sectional and cohort designs so as to address its specific objectives. In order to assess the prevalence of ZD and VAD, cross-sectional design was applied. In order to identify correlates of the deficiencies, cross-sectional study with internal comparison between “deficient” and “non deficient” groups was used. To assess the effects of prenatal ZD and VAD on birthweight, cohort study design was employed. In the later case pregnant women were classified based on their prenatal serum zinc and vitamin A statuses and followed until birth. Birthweight was measured within 72 hours of birth.

Timeframe of the Study

The baseline cross-sectional study was conducted from January 10 to February 7 2011. Among women who were at their first or second trimester of gestation during the baseline survey, follow-up data (mainly on dietary diversity and household food insecurity levels) were gathered from April 23-28, 2011. The outcome ascertainment (birthweight measurement) was continued until October 2011.

Source and Study Population

The source population of the study were all women in rural Sidama zone who were pregnant at the time of the baseline survey; whereas, the study population were all women in 18 selected kebeles of rural Sidama zone who were pregnant at the time of the survey.

Inclusion and Exclusion Criteria

For the study aiming at assessing the prevalence and correlates of ZD and VAD, all pregnant women who were the usual residents of the 18 study kebeles and who were proven to be pregnant using pregnancy urine test were eligible subjects.

For the study aiming at assessing the effects of prenatal ZD and VAD on birthweight, pregnant women whose zinc and VA statuses were determined at their second or third trimester and who later gave singleton live-births were eligible. Women whose exposure status was determined at their first trimester were excluded as the fetal weight gain during the first trimester is known to be minimal (239). Further, newborns whose weight was measured after 72 hours of birth were also excluded. The flowchart of the cohort study from exposure assessment to outcome ascertainment is depicted in Figure 3.

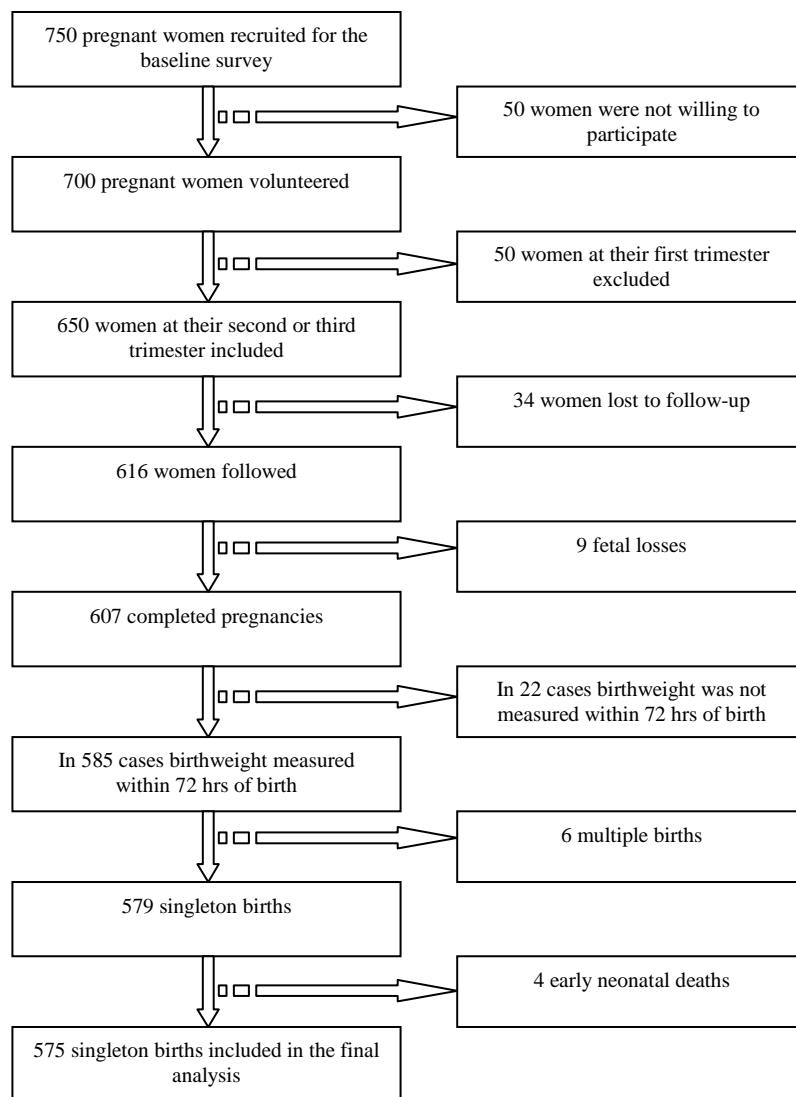


Figure 3: Flowchart of the cohort from exposure assessment to outcome ascertainment.

Sample Size

The following single proportion sample size calculation formula was applied to compute optimal sample size for estimating the prevalences of ZD, VAD and LBW. The computation was made with the inputs of 95% confidence level ($Z_{\alpha/2}=1.96$) and 5% margin of error (d). The expected prevalences (P) were taken from earlier studies conducted in the locality (18) or elsewhere in the country (183). Since the study involved multi-stage sampling technique, in the sample size calculation a design effect (DE) of 2 was considered. Further 10-15% contingency was added for the sake of accommodating possibilities of non-response or loss to follow-up.

$$n = \frac{Z_{\alpha/2}^2 P(1-P)}{d^2} \times DE$$

As noted in Table 1, sample size of 682 pregnant women would suffice to estimate the prevalences of ZD and VAD. However, in the baseline survey 750 pregnant women were recruited so as to maximize the sample size for the analytic components of the study. On the other hand, in order to determine the prevalence of LBW, 565 pregnant women who satisfied the aforementioned inclusion criteria were required; nevertheless, 575 pregnant women were available for the study.

Table 1: Sample size required for determining the prevalences of ZD, VAD and LBW.

Objectives	Z	d	DE	Contingency	P	Required sample size
Prevalence ZD	1.96	5%	2	10% non-response	72% ⁽¹⁸⁾	682
Prevalence of VAD	1.96	5%	2	10% non-response	27% ⁽¹⁸⁾	666
Prevalence of LBW	1.96	5%	2	15% loss to follow-up	20% ⁽¹⁸³⁾	565

The adequacy of the available sample size for the assessment of correlates of ZD and VAD was evaluated using the following double proportion sample size calculation formula where $Z_{\alpha/2}$ is the confidence level, Z_{β} is the power of the study, r is the ratio between “not ill” and “ill” subjects, P_1 and P_2 are the expected prevalences of the exposures in “not ill” and “ill” subjects and P is the weighted pooled proportion for P_1 and P_2 . P_2 was calculated based on P_1 and Odds Ratio (OR) for the association between the exposures and outcomes of interest.

$$n = \frac{(Z_{\frac{\alpha}{2}} \sqrt{(1 + \frac{1}{r})P(1-P)} + Z_{\beta} \sqrt{P_1(1-P_1) + \frac{P_2(1-P_2)}{r}})^2}{(P_1 - P_2)^2}$$

The computation was made via STATCALC application of EPI-Info Version 3.5.1 with the inputs of 95% confidence level, 80% power and one to one ratio between “ill” and “not-ill” groups. Expected prevalence of the exposures in “not-ill” group and OR for the association between the exposures and outcomes were taken from previous studies conducted in the country or elsewhere (Table 2). Accordingly, the existing numbers of “ill” and “not-ill” subjects in the dataset for the two deficiencies under consideration were judged to be adequate.

Table 2: Sample size calculation based on double proportion formula for investigating correlates of prenatal ZD and VAD.

Risk factors	Expected <i>P</i> of the factor in “not-ill” group	Ill : Not-ill	OR	Required sample size	
				Ill	Not-ill
Correlates of ZD					
Old maternal age ¹	31.0% ⁽⁶⁵⁾	1:1	1.61	317	317
3 rd trimester gestation ²	46.0% ⁽⁶⁵⁾	1:1	0.46	123	123
Multiparty ³	40.4% ⁽²⁴⁰⁾	1:1	3.07	58	58
Correlates of VAD					
Grand multiparty ⁴	66.6% ⁽⁴⁾	1:1	1.80	248	248
Low economic status ⁵	50.9% ⁽⁴⁾	1:1	2.69	79	79
Illiteracy ⁶	89.2% ⁽⁴⁾	1:1	3.22	230	230

1. Maternal age “>30 years” compared to “≤30 years”
2. “3rd trimester” compared to “2nd trimester”
3. “Multiparas” compared to “nulliparas”
4. Parity of “five or more” compared to parity of “one”
5. “Poorest” wealth index compared to “richest” wealth index
6. “Illiterate” compared to “beyond primary level of education”

The adequacy of the available sample size for investigating the effects of prenatal ZD and VAD on LBW was also assessed using the aforementioned double proportion formula. The power calculation was made using STATA/SE 11.0 with the inputs of 95% confidence level, 90% power and 1:1 ratio between exposed and non-exposed subjects. Expected prevalences of LBW in exposed and non-exposed subjects were taken from studies conducted abroad (37, 115). Ultimately the available sample size was judged to be adequate.

Table 3: Sample size calculation for investigating the effects of prenatal zinc and vitamin A deficiency on birthweight.

Exposures	Expected prevalence of LBW		Required sample size	
	Exposed	Non-exposed	Exposed	Non-exposed
Vitamin A deficiency	46.0% ⁽³⁷⁾	23.0% ⁽³⁷⁾	97	97
Zinc deficiency	13.5% ⁽¹¹⁵⁾	3.1% ⁽¹¹⁵⁾	165	165

Sampling Method

Initially all the rural kebeles in the zone (total of 456 kebeles) were listed and stratified into the three agro-ecological zones namely lowlands, midlands and highlands. The total sample size was divided to each of the three strata proportionally to their population size (20%, 50% and 30% respectively). From each stratum, 6 kebeles, a total of 18 kebeles, were selected using simple random sampling technique. Once again the sample size for each stratum was distributed to the six kebeles proportionally to their population size. In each of the selected kebeles voluntary Community Health Promoters (CHPs) carried out house-to-house enumeration to identify potential pregnant women. Presumptive symptoms of pregnancy (ammenoria and/or increased uterine size) with subsequent pregnancy urine test were used to diagnose pregnancy. Eventually 750 subjects were selected using systematic random sampling technique (Figure 4).

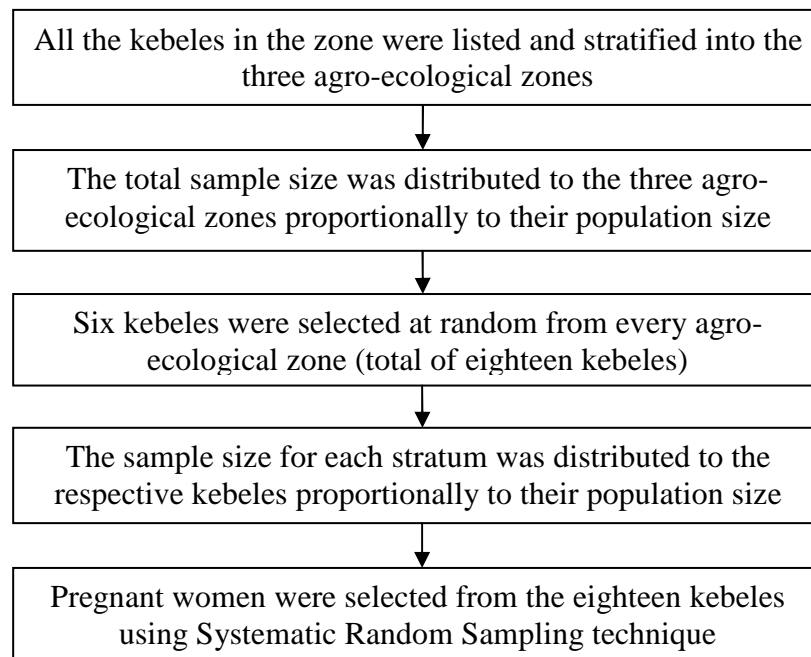


Figure 4: Schematic presentation of the sampling method of the study.

Data Collection Method

Data collection tool

During the baseline survey a structured questionnaire (Annex 1 and 2) was used to assess potential socio-demographic, economic, reproductive, dietary, medical and health care related correlates of ZD and VAD. The section of the questionnaire which deals with socio-demographic and economic information was taken from standard DHS questionnaire with minimal modification. The parts of the questionnaire used to assess the Dietary Diversity (DD) and household food insecurity levels were adopted from Food and Nutrition Technical Assistance (FANTA) indicator guide for Household Dietary Diversity Score (HDDS) (241) and Household Food Insecurity Access Scale (HFIAS) (242), respectively. Other sections were developed by the Principal Investigator (PI) in accordance with the conceptual framework of the study. The tool was developed in English and later translated and finalized in Amharic.

HDDS quantifies DD based on one day 24 hours recall method. Respondents were asked whether they had taken any food from predefined 12 food categories in the previous day of the survey. Accordingly, the Dietary Diversity Score (DDS) was computed out of 12. The HFIAS appraises the occurrence of nine food insecurity related events in the household in the preceding 4 weeks of the survey. The reporting of an event earned a score of 1 while the opposite earned 0. Accordingly, the scale was computed out of a total score of 9. The higher the HFIAS score the more serious the household food insecurity problem, and the vice versa.

Pretesting, validity and reliability assessment

The content validity of the tool was assessed against the conceptual framework of the study by five relevant professionals. In order to customize and adopt the tool to the local situation, pretesting was conducted among 33 pregnant women in Loke kebele, which is one of the rural kebeles of Sidama zone. In accordance with the findings of the pretest, minor modifications were made to the questionnaire. The reliability of the tool was assessed using test-retest method. In this case, the tool was re-administered to same group of respondents included in the pilot survey by similar data collectors after four days from the initial survey. Then the levels of agreement between the responses for various key questions in the first and second tests were statistically

compared. Pearson correlation coefficient was used to compare continuous variables whereas Kappa coefficient applied for categorical variables. Items scoring below 0.7 in either of the coefficients were removed or revised. The kebele used for the pretest and reliability assessment was excluded from the main study in order to avoid potential community fatigue.

Recruitment and training of personnel

During the baseline survey, data were collected using three trained female diploma holder clinical nurses. The supervision was made by a health officer and the PI. The recruitment of data collectors was made based on their academic qualification, work experience and language fluency (Sidamigna and Amharic languages). A six days training was offered to the data collectors by the PI using a structured training manual. The training included orientation on principles of data collection, ethics of data collection, line by line discussion on the tool, procedures of relevant anthropometric measurements, standardized presentation of the questionnaire in Sidamigna language, in-class mock interviews, and field practices.

Baseline data collection

The baseline data were gathered from January 10 to February 7 2011. Data were collected in private setting within the compound of the nearby health posts. The questionnaires were administered using Sidamigna language. In average each interview took 25-35 minutes and every data collector conducted 10 interviews per day. Pallor, Bitot spot and Goitor examinations and fundal height and blood pressure measurements were carried out by an experienced public health officer. Resting blood pressure was measured in duplicate using Mdf 800 ® mercury Sphygmo-manometer. The measurement was taken on the right arm after subjects had been in a sitting position for a minimum of 25 minutes. The mean of the two measurements was ultimately registered. The altitude of the study kebeles was measured at the center of the kebeles using Magilan ® GPS system.

Anthropometric measurement and gestational age determination

Maternal anthropometric measurements were taken during the baseline survey. Weight was measured using pretested and calibrated digital Seca® scale and recorded to the nearest 0.1 kg. Maternal height was measured to the nearest 0.1 cm using a wooden stadiometer with sliding head bar. MUAC was also measured to the nearest 0.1 cm using MUAC tape. Anthropometric measurements were taken meticulously following standard procedures. Weight was measured without shoes and heavy clothing. Height was measured without shoes, standing erect, feet together, heels, buttocks, and occiput touching the stadiometer and looking straight ahead. MUAC was taken on the middle left arm at relaxed position, without any clothing and with optimal tape tension. All anthropometric measurements were taken twice and the average of the two observations was registered. At times when unexpected discrepancies were observed additional measurements were taken. Gestational age was determined based on Last Normal Menstrual Period (LNMP). At times when the respondents failed to remember their LNMP, estimation was made based on fundal height.

Blood sample collection, serum extraction, transportation and storage

Blood sample collection and processing were done by an experienced B.Sc. degree holder laboratory technician. Six ml of blood was collected from antecubital vein using plain and closed SARSTEDT Monovette® blood collection system and SARSTEDT® butterfly stainless steel needles. In order to avoid hemolysis, serum was extracted in the health posts within 40 minutes of sample collection using a portable diesel generator and a centrifuge. After sample collection, the blood was allowed to clot for 20 minutes in a closed icebox and centrifuged at 3000 RPM for 10 minutes. Then serum was extracted and transferred into labeled screw-top vials. During this time few obviously hemolyzed samples were identified and discarded. In the entire process, the samples were protected from dust and direct light. In the field the samples were kept and transported in icebox. The same day they were stored frozen at -20°C. At the end of the survey the samples were transported in icebox to the Ethiopian Health and Nutrition Research Institute (EHNRI) laboratory and kept frozen at -20°C until analyzed.

Birth identification system and birthweight measurement

Birth identification and birthweight measurements were carried out by involving local Community Health Promoters (CHPs) and Health Extension Workers (HEWs), respectively. Prior to the baseline survey, a two days training was offered to 31 HEWs involved in the study. The training included orientation on the purpose of the study, demonstration on how to calibrate the weighing scale and measure newborns' weight, discussion on how to work with the CHPs and orientation on how to fill the checklist (annex 3 and 4). An orientation has also been given to CHPs at their respective kebeles on how to identify births and how to network with HEWs.

Depending on the population size of the study kebeles, in every kebele 30-45 pregnant women were followed until birth and 9-12 CHPs were deployed to identify and report the occurrence of births to the respective HEWs. In order to enhance the motivation and adherence of the CHPs, an incentive based system was applied. All pregnant women were assigned to the CHPs according to their geographical proximity. Once the occurrence of birth is reported, birthweight was measured at home within 72 hours of birth by the respective HEWs. Prior to every measurement the scales were calibrated. Birthweight was measured without any cloth using Docbel BRAUN® scale and registered to the nearest 100g. In addition, information pertaining to labor and postpartum conceptions of the newborn were registered using the checklist.

The activities on birthweight measurement were supervised every 3-4 weeks by three B.Sc. degree holder supervisors. The supervisors made random post-measurement visit to two-fifth of the mothers reported to have birth so as to verify that the measurement had been carried out in the specified time.

Laboratory analyses:

Serum zinc determination: Serum zinc level was determined within two months of sample collection. The analysis was done at EHNRI by experienced laboratory technicians. The PI was actively involved in sample preparation. The analysis was made using Varian SpectrAA ® Flame Atomic Absorption Spectrometer (AAS) in accordance with the manufacturer's operating manual. Samples were analyzed at wave length of 213.9 nm, with lamp current of 5.0 mA and slit width of 1.0 nm. Initially, standards of 0.5, 1.0, 1.5 and 2.0 ppm were prepared by diluting 1000 PPM zinc standard in an appropriate volume of 6% butanol. Then the standards were used for calibration. The samples were prepared by diluting well vortexed 200 µl serum in 2 ml of 6%

butanol. After the AAS was optimized, the zinc concentration of each sample was read three times and the average was recorded. The analysis was repeated for samples which yielded Relative Standard Deviation (RSD) exceeding 3%. Samples were analyzed in a batch of 25. A control sample with known concentration and multiple blank samples were run with every batch. At times when unexpected concentration is reported either for the control or blank samples, sample preparation and analyses were repeated for the whole batch. The AAS system was flushed with 6% butanol after every analysis.

Serum retinol determination: Serum retinol concentration was determined at EHNRI within three months of sample collection using Shimadzu® High Performance Liquid Chromatography (HPLC). The analysis was done following standard procedures (243). The sample preparation was done by the PI whereas the standards preparation and analysis were carried by an experienced laboratory technician. Initially 5, 10, 20, 40 and 60 µg/dl of retinol standards were prepared. Then 250 µl ethanol and 250 µl retinol acetate solutions were added into 250 µl of serum. The mixture was vortexed for 45 seconds, 2 ml of hexane was added and centrifuged at 3000 RPM for 15 minutes. Subsequently the upper hexane layer was carefully removed using a micropipette and transferred into a second tube. Again another 2 ml of hexane was added to the first tube and the process of extraction was repeated. Finally the hexane was evaporated with a stream of pure dry nitrogen and the residual was reconstituted in 800 µl methanol. Ultimately the HPLC was calibrated using the standards and retinol was detected with an ultraviolet detector at 325 nm. In order to assure the quality of analysis, two controls with known retinol concentrations were assayed with every batch of samples.

Hemoglobin and serum ferritin determination: Blood hemoglobin level was determined in the field instantly after sample collection using HemoCue Hb 301®. The analysis was done using a drop of whole blood pipetted from gently shaken venous blood sample. After analysis, hemoglobin values were adjusted for altitude according to the recommendation of Center for Disease Prevention and Control (CDC) (244). On the other hand, serum ferritin concentration was determined at EHNRI within two months of sample collection using fully automated Roche-Elecsys® Chemistry Analyzer. A volume of 250 µl of serum was used for the analysis. Neither of the tests requires special sample preparation.

C - Reactive Protein (CRP) test: CRP level was determined qualitatively using latex HumaTex® kit following standard procedures (245). The more sensitive quantitative technique was opted out due to its expensive cost and limited local availability. CRP test was conducted by the PI. Initially a drop of CRP latex reagent was added into 40 µl non-diluted serum then the mixture was gently rotated at 100 RPM for 2 minutes using an automatic shaker. A distinctively visible agglutination was indicative of positive CRP result (CRP > 5 mg/dl). In conjunction with every batch of test comprising 50 samples, positive and negative control tests were carried out in order to assure the quality of the analysis.

Data Management and Analyses

Data entry, screening and management

Data entry, screening and analyses were carried out by the PI primarily using SPSS 19.0 for windows. After an entry is completed, its correctness was rechecked by going through the questionnaire again. Prior to analysis the data were screened for missing, out of range and outlier values. Missing and out of range values were tracked by examining the frequency distribution of the variables. Such entries were validated against the raw data and in case of entry errors corrections were made accordingly. In all of the variables actual missing values were very few and random hence they were left as they were. The presence of outliers in the pertinent continuous variables was diagnosed using Box and Whiskers plot. Extreme values were trimmed to the nearest acceptable value.

The normality of the distribution of key continues variables (serum zinc, VA, ferritin, hemoglobin and birthweight) was assessed using various options including statistical tests (Kolmogorov-Smirnov and Shapiro-Wilk tests) and visual evaluation of histogram and probability plots. All the aforementioned variables, except serum ferritin, were normally distributed. Ferritin exhibited positively skewed distribution. Accordingly logarithmic transformation was applied to switch it into a normal distribution.

Basic data analysis

Data description was made using mean, median, Standard Deviation (SD), frequency, percentage and graphs. Estimates of population parameters were presented with 95% Confidence Interval (CI). In every application of inferential statistics *P* value of 0.05 was taken as significance level. Independent t-test and one-way Analysis of Variance (ANOVA) with Tukey's post-hoc test were used to compare serum zinc, VA, and birthweight across different categories of the independent variables. Two of the major assumptions of ANOVA (homoscedasticity and normality of the dependent variable) were checked to be satisfied. Correlation analysis was applied to measure the strength association between two numeric variables. Prior to application, the major assumptions of the Pearsonian correlation (normality of the variables and linearity of association) were also checked.

Principal Component Analysis (PCA)

Wealth index was computed using PCA as a composite indicator of living standard based on ownership of selected household assets, size of agricultural land, quantity of livestock, materials used for housing construction, and ownership of improved water and sanitation facilities. Initially 23 variables were considered for the analysis. In confirmation of the assumptions of PCA (246), the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.76 and Bartlett's test of sphericity was significant. During analysis three variables (use of electricity service, number of chickens and size of agricultural land) were dropped as their communality scores were less than 50%. Ultimately, seven principal components having eigenvalues greater than one were identified (247). The components explained 61.1% of the total variance which was above the recommended minimum value of 60% (246). Wealth index values were calculated by summing up the scores for the seven components. Ultimately, the five categories (poorest, poorer, middle, richer, and richest) were generated by splitting the wealth index values into 5 equal classes.

PCA was also used for the purpose of data reduction pertaining to maternal workload. Initially the frequencies of 10 distinct laborious activities in the community in the preceding week of the survey were included in the analysis. In confirmation of the assumptions of PCA, the KMO measure of sampling adequacy was 0.70 and Bartlett's test of sphericity was significant. The variables had communality score greater than 50%, hence they were kept in the analysis. Finally, four principal components were identified. In combination, the generated components explained 68.7% of the total variance. Ultimately, five categories of maternal workload (lowest, lower, middle, higher and highest) were generated.

Logistic regression analysis

Binary logistic regression analysis was applied to control potential confounders and to identify factors associated with of ZD and VAD. The dependent variables were dichotomized based on standard cutoff points. Initially every variable was independently entered into the models. Variables significant at bivariate analysis level were subsequently considered for the multivariate analysis. In multivariate analysis, variables were entered using the “enter” method. Logistic models were constructed in levels according to the conceptual framework of the study. For instance, in the analyses of correlates of ZD and VAD, distal factors like agro-ecological zone and socio-demographic factors were included in a model whereas other more proximate factors were incorporated in a different model. Before analysis, the major assumptions of logistic regression (absence of multicollinearity and interaction among independent variables) were checked to be satisfied. The fitness of logistic regression models were assessed using Hosmer-Lemeshow statistic. The outputs of simple and multiple logistic models were presented in Crude Odds Ratio (COR) and Adjusted Odds Ratio (AOR), with their respective 95% CI.

Linear regression analysis

Linear regression analysis was also applied to control potential confounders and to identify correlates of birthweight and, prenatal zinc and retinol levels. Alike the logistic model, primarily every covariate was entered independently into the model and only significant ones were exported for multivariate analysis. Variables were entered in levels according to the conceptual framework of the study. The major assumptions of the analysis (normality, homoscedasticity and independence error terms, linearity between dependent and independent variables and absence of

multicollinearity) were not violated. The assumption of normality of error terms was checked via statistical normality tests and visual evaluation of histogram and probability plots. For a given covariate multicollinearity was considered to be absent when its Variance Inflation Factor (VIF) is below 10 (248). Assumptions of linearity, homoscedasticity and independence of error terms were visually evaluated using residual plots. The fitness of linear regression models were assessed using adjusted r-squared and F test statistic values. The outputs of the analyses were given in unstandardized and standardized regression coefficients.

Log-binomial regression analysis

Analysis of determinants of LBW was carried out using log-binomial model. The analysis was done using STATA/SE 11.0. The model was preferred as it directly estimates Relative Risk (RR). Working with RR is more appropriate due to the longitudinal nature of the data. In addition, as the prevalence of LBW was above 10%, use of OR as a measure of association can potentially result in overestimation of risk (249). In the log-binomial model, in accordance with the framework of Kramer (34), the independent variables were entered separately in two blocks of indirect and direct factors. The indirect factors comprised agro-ecological zone and socio-demographic factors where as the direct factors included ZD, VAD, maternal thinness and stunting, CRP status, newborn sex, maternal age and primiparity. The goodness-of-fit of the model was assessed using Pearson chi-square and Deviance tests. The outputs of the analyses were given in Crude Relative Risk (CRR) and Adjusted Relative Risk (ARR).

Interaction assessment

Potential interaction of zinc and VA deficiencies in causing LBW was measured on additive scale. The additive scale was preferred as it is known to have better correlation with biological interaction (250, 251). The Synergy Index (SI) was calculated based on the original Rothman's formula (251). The 95% Confidence Interval (CI) for SI was computed manually following the recommendations of Hosmer and Lemeshow (252).

Dependent and Independent Variables

The set of dependent and independent variables were different across the papers included in the dissertation. In the assessment of the correlates of prenatal ZD and VAD, the dependent variables were serum retinol level/VAD and serum zinc levels/ZD. While the independent variables were agro-ecological zone, kebele of residence, maternal age, gestational trimester, household economic status, maternal educational status, maternal employment, parity, birth interval, MUAC, household food insecurity level, dietary diversity level, consumption of animal source foods, type of staple diet, frequency of coffee consumption, maternal workload, distance from the nearby health facility, frequency of ANC visit, history of prenatal iron supplementation, history of nutrition education during pregnancy and CRP status.

In the evaluation of the effects of ZD and VAD on birthweight, birthweight/LBW was the dependent variable whereas the major independent variables were zinc and retinol levels. Other independent variables of interest include maternal literacy, maternal employment, agro-ecological zone, frequency of ANC visit, diastolic blood pressure during pregnancy, type of staple diet, distance from the nearby health facility, maternal thinness, maternal stunting, CRP status, newborn sex, maternal age and primiparity.

Quality Assurance Mechanisms

Starting from tool designing to data analysis, various actions were instated to assure the quality of the research. The questionnaire was prepared using simple and easily understandable Amharic language and administered using the local language via data collectors fluent in both languages. The instrument was pretested in the locality and appropriate customization and modifications had been made. The reliability and content validity of the tool have also been evaluated. Accordingly items with lower reliability coefficients were amended or removed.

Data were gathered using appropriately qualified and experienced data collectors. During the baseline survey an intensive six days training including orientation, demonstration and field practice has been offered. Strict data collection supervision was instated by the supervisors. On daily basis, the PI reviewed every filled questionnaire and provided proper feedback to the data collectors. In addition, regular evening sessions were arranged for the sake of experience sharing purpose. Maternal and newborn anthropometric measurements were taken using calibrated and pretested scales. Birthweight measurement was done by properly trained HEWs. Random post-measurement visit was made to two-fifth of the mothers reported to have birth so as to verifying that the measurement had been carried out in the specified time.

Data entry, screening and management were exclusively done by the PI. In order to avoid potential errors every entry has been counterchecked. After the completion of the entry, the data have been screened for missing, out of range and outlier values following standard procedures. The normality of pertinent continuous variables has been well assessed. The assumptions and fitness of statistical models have been checked following standard procedures.

Blood sample collection and laboratory analyses followed standard procedures. Biological samples were collected using trace element free tubes. For the sake of avoiding risk of hemolysis and contamination, serum extraction was carried out in the field within 40 minutes of sample collection. The samples were transported and stored in confirmation of international practice. The laboratory analyses were conducted at EHNRI using appropriately qualified personnel. The quality of the laboratory analysis was assured by the use of proper controls and blank samples. The PI involved in the entire process of the laboratory analyses.

Ethical Considerations

The study was conducted in confirmation of national and international ethical guidelines for biomedical research involving human subjects. Ethical clearance was granted by the Institutional Review Board (IRB) of College of Health Science, Addis Ababa University (AAU). Prior to the study its purpose was communicated and permission was taken from SNNPR health bureau, Sidama zone health department, respective woreda health offices and kebele administrations. Regional health bureau and zonal health department representatives were also invited to supervise the data collection process.

Participation of the study subjects was entirely on voluntary basis. Informed written consent (signed or verified by finger print) was taken from the study subjects using the annexed consent and information forms (Annex 1 and 2). The consent was secured after the purposes of the study, the rights of participants, potential benefits and harms of the study etc were thoroughly and privately communicated. As the study involved “no more than the minimal risk” permission was not sought after the husbands. The study did not involve any undue financial or non financial inducement. The subjects were not persuaded or influenced in anyway by local officials or health service providers to take part in the study.

All the necessary measures were taken to eliminate potential risks associated with study. Injection safety was up to the international standards. Clean gloves, sterile and use-and-throw blood collection systems and appropriate disinfectants were universally used. As stated in the information form, only 6 ml of blood was drawn from every subject. The blood samples have not and will not be used apart from the tests required in the protocol and they will be discarded accordingly after the completion of the study. As the study was not interventional, it did not involve any supplementation or dietary modification. All the information provided by the study subjects were kept confidential.

Utmost effort was made to maximize the beneficence aspect of the study. Anemic women were provided iron-folate supplementation and women with visible goiter were offered iodized salt. Nutrition education was available to all subjects on the manifestation, consequences and prevention of the common nutritional deficiencies and on the dimensions of optimal maternal nutrition. Women were advised and encouraged to initiate ANC and to give birth in health institutions. After delivery HEWs had provided Postnatal Care (PNC) to the mothers and newborns. As the existing national regulation does not allow the administration of zinc supplement to pregnant women, zinc deficient women were not supplemented. In order to maximize the benefit of the study to the community, the findings of study have been selectively disclosed to the local authorities so that suitable interventions can be instated.

Methods for Systematic Review and Meta-analysis

The dissertation included a systematic review intended to evaluate the effect of prenatal zinc supplementation on birthweight. The methods of the review are given as follows.

Study Design:

This is a systematic review which includes Meta-analysis based on Literature (MAL).

Inclusion and Exclusion Criteria:

The inclusion criterion was set primarily on the design of the original studies. For the sake of assuring the quality of the analysis, the review was delimited to RCTs. Exclusion criteria were defined based on multiple attributes, including low dose of supplementation (less than 15 mg/day), unavailability of vital information in the articles and low level of compliance (<70%) for the supplement. Further, papers written in English were only considered for the analysis. The year of publication was not among the exclusion criteria as the effect under consideration is not known to be time dependent.

Search Strategy:

Studies were mainly identified through web-based search including descendent and ancestor search techniques. The meta-analysis did not involve any manual search or contacts with authors. Key search terms were “zinc supplementation and birthweight” and “zinc supplementation and birth outcomes”. The search was primarily made within PubMed/MEDLINE and Cochrane databases. Further studies were identified using the Google Scholar search engine. Initially more than 50 studies were identified. However based on the evaluation of the abstracts 21 (20 published and 1 PhD dissertation) studies were found to be relevant for the analysis.

Evaluation of Studies:

The quality of the studies was assessed based on various parameters including level of compliance for the supplementation (>70% compliance), and the comparability of the arms based on potential confounders. In addition the appropriateness of the studies was evaluated based on the availability and extractability of the aforementioned vital information for the analysis. Three of the 21 RCTs, conducted in Germany (253), USA (107) and Denmark (254), were excluded as vital information were lacking or unextractable. A study in South Africa was precluded since it used very low dose (4.3-12.9 mg/day) supplement (255). Hence, the analysis was done with the remaining 17 studies.

Data Extraction:

From each of the studies included, information on total number of zinc supplemented and control groups, number of low birthweight babies (<2500 gm) in both arms, mean birthweight and SD for both arms, nature of the population, dose and duration of supplementation were extracted. Information on other potential confounding factors has not been abstracted as they were not uniformly presented across the original studies.

Data Analysis:

The description of original studies was made using frequency and forest plot. Heterogeneity assessment, based on Cochran Q test statistic, demonstrated random variation ($P=0.09$) among the studies. Hence, random effect model was used to compute the pooled estimated. Between studies variation was assessed using DerSimonian and Laird's (DL) estimator. Stratified analysis was made to evaluate any differential effects based of dose of supplementation (optimal or high dose), type of the study (community or institutional based) and development status of the country where the study was conducted. Data analysis was carried out using Metaeasy 1.0 add-in for MS Excel. As relatively fewer articles were included in the analysis, publication bias was not assessed using Funnel plot.

Operational Definitions

1. Zinc deficiency: Serum zinc level of less than 56 µgm/dl (8.6 µmol/l) during the first trimester, or less than 50 µgm/dl (7.6 µmol/l) during the second or third trimester (86).
2. Anemia: Hemoglobin level of less than 11.0 g/dl during the first or third trimester or less than 10.5 g/dl during the second trimester (256).
3. Vitamin A deficiency: Serum retinol concentration less than 0.7 µmol/l irrespective of the gestational age (16).
4. Marginal vitamin A status: Serum retinol concentration of 0.7 to 1.05 µmol/l irrespective of the gestational trimester (16).
5. Iron deficiency: Serum ferritin less than 15 mg/l (257).
6. Iron deficiency anemia: Concurrence of anemia with serum ferritin less than 15 mg/l (258).
7. Maternal severe acute malnutrition: Pregnant women with MUAC less than 220 mm (259).
8. Fasting: No intake of food or beverage in the preceding 8 hours of the sample collection (86).
9. Low dietary diversity: Diet diversity score less than or equal to three (260).
10. Medium dietary diversity: Diet diversity score of four or five (260).
11. High dietary diversity: Diet diversity score greater than or equal to six (260).
12. Lowlands: Geographical area located below 1750 meters above sea level.
13. Midlands: Geographical area located 1750 to 2300 meters above sea level.
14. Highlands: Geographical area located above 2300 meters above sea level.
15. Birthweight: Weight of the baby as measured within the first 72 hours of birth.
16. LBW: Birthweight less than 2.5 kgs as measured within the first 72 hours of birth.
17. Maternal stunting: Pregnant women with height less than 145cm (261).
18. Birth interval: The number of months between the month of the most recent pregnancy ended and the estimated month of birth of the index pregnancy (262).
19. Optimal birth interval: Birth interval longer than (or equal to) 24 months.
20. Short birth interval: Birth interval shorter than 24 months.
21. Gestational week: Gestational age in weeks as measured by LNMP or fundal height.
22. Positive CRP test: CRP greater or equal to 5 mg/dl.

Tabular Summary of the Methods Applied in the Dissertation

Table 4: Summary of the methods applied in the dissertation

No	Objectives	Study design	Study subjects	Sample size required	Data collection method	Data analyses
1	To determine the prevalence of prenatal ZD	Cross-sectional	Pregnant women at any gestational trimester	682 pregnant women	Serum zinc level determined using AAS	Descriptive analysis
2	To determine the prevalence of prenatal VAD	Cross-sectional	Pregnant women at any gestational trimester	666 pregnant women	Serum retinol level determined using HPLC	Descriptive analysis
3	To identify correlates of zinc status during pregnancy	Cross-sectional comparative	Pregnant women at any gestational trimester	317 zinc deficient and 317 normal pregnant women	Information about potential correlates collected using a structured questionnaire	Linear and logistic regression analyses
4	To identify correlates of VA status during pregnancy	Cross-sectional comparative	Pregnant women at any gestational trimester	248 VA deficient and 248 normal pregnant women	Information about potential correlates collected using a structured questionnaire	Linear and logistic regression analyses
5	To determine the prevalence of LBW	Cross-sectional	Singleton births	565 newborns	Birthweight measured within 72 hrs of birth	Descriptive analysis
6	To evaluate effect of prenatal ZD on birthweight	Prospective cohort	Pregnant women whose zinc status were determined in the 2 nd or 3 rd gestational trimester	165 zinc deficient and 165 normal pregnant women	Zinc status determined in the 2 nd or 3 rd trimester and birthweight measured within 72 hrs of birth	Linear and log-binomial regression analyses
7	To evaluate effect of prenatal VA deficiency on birthweight	Prospective cohort	Women whose VA status were determined in the 2 nd or 3 rd gestational trimester	97 VA deficient and 97 normal pregnant women	VA status determined in the 2 nd or 3 rd trimester and birthweight measured within 72 hrs of birth	Linear and log-binomial regression analyses
8	To assess the effect of prenatal zinc supplementation on birthweight	Meta-analysis based on literature	RCTs conducted in the world on the effect of zinc supplementation on birthweight	RCTs which satisfy predefined inclusion criteria	Web based search of literatures	Meta-analysis based on fixed and random effect models

V. Results

Socio-demographic Information

Of 750 pregnant women sampled at the baseline study, 700 volunteered to participate. Consequently, the response rate of the survey was 93.3%. The study participants were selected across 18 rural kebeles with altitude ASL ranging from 1584 to 2763 meters. Comparable to the natural composition of the population, about half, 357 (51.0%), of the subjects were recruited from the midlands; whereas, 187 (26.7%) and 156 (22.3%) were from the highlands and lowlands, respectively.

During the baseline survey only few, 50 (7.2%), of the study subjects were at their first gestational trimester while the predominating 347 (49.6%) and 303 (43.3%) were at their second and third trimesters, respectively. The mean age (\pm standard deviation) of the respondents was 28.5 (\pm 5.5) years and more than three-quarters, 542 (77.4%), were between 20 to 35 years of age. The median parity was 2 and ranged from 0-9. About 116 (16.6%) and 77 (11.0%) of the respondents were nulliparas and grand multiparas, correspondingly.

In terms of educational status, 462 (66.0%), of the subjects were illiterates and 127 (18.2%) had informal or primarily level of education. As to occupation is concerned, 605 (86.4%) identified themselves as housewives/farmers and merely 95 (13.7%) were regularly involved in Income Generating Activities (IGAs) like petty trade and daily labor. The vast majority, 679 (97.0%) and 596 (85.1%), were Sidama in ethnicity and Protestant Christians in religion (Table 5).

The median household income in a typical month was 200 birr (11.3 United States Dollar) and ranged from 50-8000 birr. The average household size was 4.3 (\pm 1.8). Nearly all, 687 (98.1%), of the households represented owned agricultural land. The average size of the land per household was 0.38 (\pm 0.19) hectares. On average every household owned 2.0 chickens, 1.3 cows/oxen, 1.0 sheep/goats and 0.4 horses/donkeys/mules.

Table 5: Socio-demographic and economic characteristics of study participants, rural Sidama, Southern Ethiopia, Feb 2011.

Variables	Frequency (n=700)	Percentage
Gestational trimester		
First	50	7.2
Second	347	49.6
Third	303	43.3
Age (years)		
15-19	41	5.9
20-24	117	16.7
25-29	256	36.6
30-34	169	24.1
35-39	96	13.7
≥ 40	21	3.0
Parity		
0	116	16.6
1-2	276	39.4
3-4	231	33.0
5	77	11.0
Educational status		
Illiterate	462	66.0
Informal education	4	0.6
1 st - 4 th grade	123	17.6
5 th - 8 th grade	97	13.9
9 th - 12 th grade	14	2
Occupation/Livelihood		
Housewife/farmer	605	86.4
Petty-trade	84	12.0
Others	11	1.6
Ethnicity		
Sidama	679	97.0
Amhara	13	1.9
Welaita	5	0.7
Others	3	0.4
Religion		
Protestant	596	85.1
Catholic	60	8.6
Muslim	30	4.3
Orthodox	14	2.0
Marital status		
Married/Living together	696	99.4
Single (not ever married)	2	0.3
Widowed	2	0.3

Prevalence of Zinc, Vitamin A and Iron Deficiencies

The mean serum zinc concentration was 52.4 (± 9.9) $\mu\text{g/dl}$ (95% CI: 51.6-53.1 $\mu\text{g/dl}$). The level declined from 57.2 (± 9.4) $\mu\text{g/dl}$ in the first trimester to 53.6 (± 9.8) and 50.1 (± 9.9) $\mu\text{g/dl}$ in the second and third trimesters, respectively. The global difference across the trimesters was significant ($F = 20.170$, $P = 0.000$). About half, 53.0% (95% CI: 49.3-56.7%), of the subjects had ZD and the prevalence at the first, second and third trimesters were 46.8, 48.5 and 58.0%, consecutively. Compared to women in the first trimester, the risk of ZD was 1.57 (95% CI: 1.29-1.91) times elevated amongst women in third trimester.

The mean serum retinol level was 0.84 (± 0.41) $\mu\text{mol/l}$ (95% CI: 0.81-0.87 $\mu\text{mol/l}$). The levels in the first, second and third trimesters were 0.97 (± 0.40), 0.89 (± 0.42) and 0.76 (± 0.40) $\mu\text{mol/l}$, respectively. The retinol level in third trimester was significantly lower than the corresponding values for the first and second trimesters ($F = 11.080$, $P = 0.000$). Overall more than one-third, 37.9% (95% CI: 34.3-41.5%), of the subjects had VAD and 28.7% (95% CI: 25.3 - 32.1%) had borderline status. Women in the third trimester had 2.59 (95% CI: 1.23-5.48) times higher risk of VAD compared to those in first trimester.

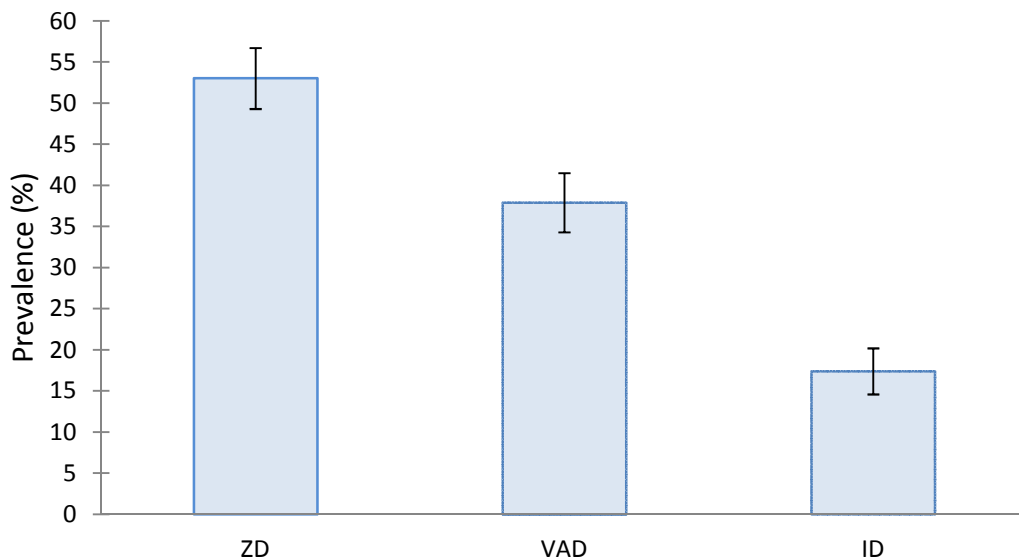


Figure 5: Prevalence of prenatal zinc, vitamin A and iron deficiencies in rural Sidama, Southern Ethiopia, Feb 2011.

The median serum ferritin concentration was 29.0 mg/l. The corresponding median values for the first, second and third trimesters were 37.3, 31.4, 26.6 mg/l, respectively. The global difference across the trimesters was statistically significant ($F=6.922$, $P=0.001$). Post-hoc test identified marginally significant lower ferritin concentration in the third trimester compared to the first trimester ($p=0.046$). About 17.4% (95% CI: 14.6-20.2%) of the subjects had ID. Nevertheless, the risk of ID was not significantly different across the three trimesters.

The interface among zinc, vitamin A and iron deficiencies was assessed. Above two-third, 492 (70.3%), of the pregnant women had at least one of the three deficiencies and merely 208 (29.7%) were free from any of the deficiencies. Approximately one-third, 230 (32.9%), had two or more concomitant deficiencies; and 36 (5.1%) had the three deficiencies. The interface among the three deficiencies is presented in the following Venn-diagram (Figure 6).

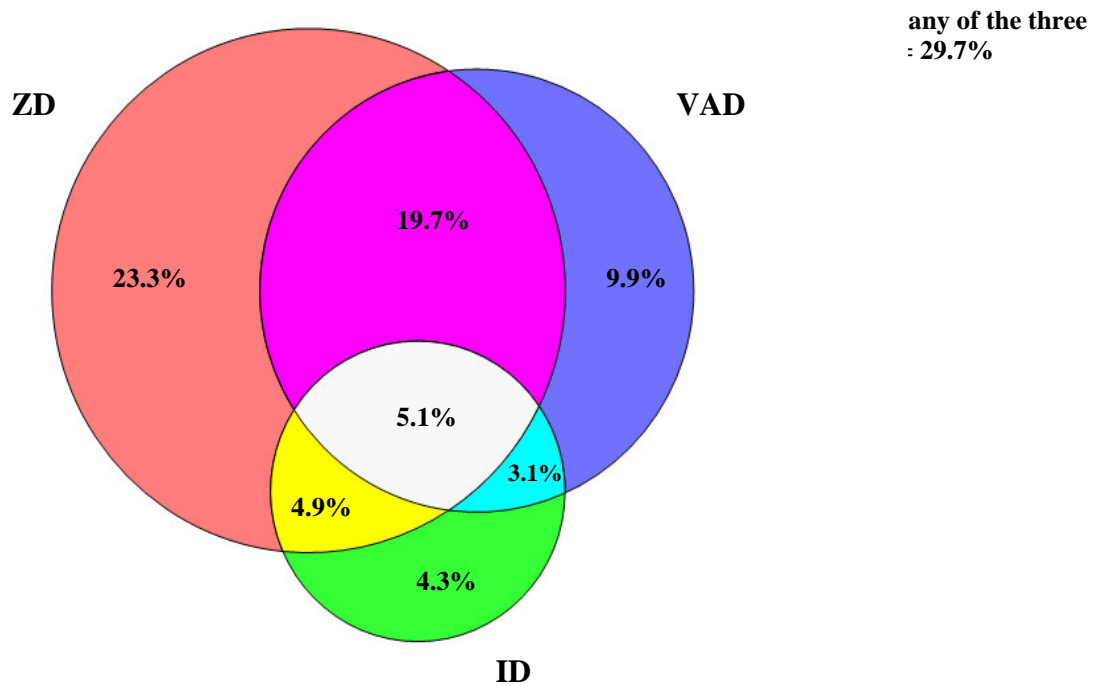


Figure 6: The interface among zinc, vitamin A and iron deficiencies during pregnancy in rural Sidama, Southern Ethiopia, Feb 2011.

Correlates of Prenatal Zinc Status

CRP Status, Fasting and Diurnal Variations

About 59 (8.4%) of the study subjects were positive for CRP test which is indicative of acute infection or inflammation. The zinc concentration in CRP positive and negative subjects were 49.8 (± 9.6) and 52.6 (± 9.9) $\mu\text{g/dl}$, respectively. The difference was statistically significant ($t=2.159$, $P=0.034$). The difference corresponds to 5.3% reduction in serum zinc concentration in CRP positive subjects.

The mean serum zinc level for 504 samples collected in the morning was 53.2 (± 9.9) $\mu\text{g/dl}$ and the analogous value for 196 samples collected in the afternoon was 50.1 (± 10.0) $\mu\text{g/dl}$. The difference was statistically significant ($t=3.755$, $P=0.000$). In multivariate linear regression model, the net disparity between samples collected in the morning and afternoon was 1.23 $\mu\text{g/dl}$ ($t=2.386$, $P=0.017$).

In current study the median time gap between the intake of the recent meal/beverage and the time of sample collection was 4 hours and ranged from 1-12 hours. About 54 (7.7%) of the study subjects fulfill the definition for “fasting”. The mean zinc concentrations among fasting subjects (52.3 \pm 9.9 $\mu\text{g/dl}$), was not significantly different from the level among non-fasting subjects (52.9 \pm 9.9 $\mu\text{g/dl}$) ($t=1.761$, $P=0.079$). Further, in a simple linear regression model, the number of hours since the recent meal/beverage was not related to serum zinc level ($t=0.370$, $P=0.712$).

Zinc Deficiency and Socio-demographic Factors

A significant inversely association was observed between maternal age and zinc status. Zinc level declines from 53.6 (± 10.2) $\mu\text{g/dl}$ in 15-24 years age group to 52.2 (± 9.9) and 51.4 (± 9.5) $\mu\text{g/dl}$ in 25-34 and 35-49 years age group, respectively ($F= 3.819$, $P=0.022$). Using multivariate linear regression model where 53.3% of the variability of serum zinc was explained, one year increment in maternal age was linked with 0.16 $\mu\text{g/dl}$ decline in zinc concentration ($t=2.410$, $p=0.016$). According to the logistic model, compared to the youngest group, women aged 25-34 and 35-49 years had 1.57 (95% CI: 1.04-2.34) and 2.18 (95% CI: 1.25-3.63) times elevated risk of ZD, respectively.

Zinc status tends to improve as maternal education advances. Zinc concentration in illiterates was 51.6 (± 9.9) $\mu\text{g/dl}$; whereas the corresponding values for women who completed 1st-4th grade and beyond 4th grade were 52.6 (± 9.5) and 54.9 (± 9.5) $\mu\text{g/dl}$, respectively. The difference was significant ($F = 4.728$, $P = 0.009$). With reference to women educated beyond 4th grade, the risk of ZD was 1.71 (95% CI: 1.09-2.60) and 1.69 (95% CI: 1.01-2.85) times higher among illiterates and women with 1st-4th grade of education, consecutively.

As depicted in Table 6, the mean zinc concentration and the risk of ZD did not show any peculiar pattern across the five wealth index quintiles. However, women's involvement in IGA appears to have affirmative influence on zinc status. A net 1.7 $\mu\text{g/dl}$ difference in serum zinc level was observed between women involved in IGA and women devoid of self income. Based on independent t-test analysis the observed difference was not significant ($t = 1.522$, $P = 0.280$). However, according to the multivariate logistic model, women devoid of self income had 1.74 (95% CI: 1.11-2.74) times increased risk of ZD.

There was no significant difference in serum zinc level across the 18 study kebeles ($F = 0.442$, $P = 0.954$). Likewise, altitude was not correlated to serum zinc level ($r = 0.041$, $P = 0.277$). The mean serum zinc level and risk of ZD were not significantly different across categories of agro-ecological zone, religion, ethnicity and husbands' educational status (Table 6).

Table 6: Various socio-demographic and economic correlates of zinc deficiency among pregnant women in rural Sidama, Feb 2011.

Variables (n=700)	Serum zinc µg/dl mean (sd)	Frequency		Crude OR (95% CI)	Adjusted OR (95% CI) **
		ZD+	ZD-		
Age (years)					
15-24	53.6 (10.2)	66	92	1 ^r	1 ^r
25-34	52.2 (9.9)	232	193	1.68 (1.14-2.47)*	1.57 (1.04-2.34)*
≥ 35	51.4 (9.5)	73	44	2.31 (1.38-3.89)*	2.18 (1.25-3.63)*
Maternal educational status					
Illiterate	51.6 (9.9)	255	207	1.74 (1.14-2.65)*	1.71 (1.09-2.60)*
1 st -4 th grade	52.6 (9.5)	70	57	1.74 (1.05-2.90)*	1.69 (1.01-2.85)*
Higher than 4 th grade	54.9 (9.5)	46	65	1 ^r	1 ^r
Involvement in IGA					
No	52.1 (9.9)	333	272	1.84 (1.18-2.86)*	1.74 (1.11-2.74)*
Yes	53.8 (10.2)	38	57	1 ^r	1 ^r
Wealth index quintiles					
Poorest	52.6 (9.6)	73	66	1.00 (0.62-1.60)	-
Poorer	51.4 (10.3)	74	54	1.24 (0.77-2.01)	-
Middle	53.1 (10.2)	78	74	0.93 (0.59-1.47)	-
Richer	51.9 (9.4)	72	68	0.96 (0.60-1.53)	-
Richest	52.7 (10.1)	74	67	1 ^r	-
Agro-ecology					
Lowland	52.9 (10.3)	105	82	0.99 (0.64-1.51)	-
Midland	52.6 (9.6)	179	178	1.25 (0.86-1.83)	-
Highland	52.9 (10.1)	87	69	1 ^r	-
Husband's educational status					
Illiterate	51.4 (9.9)	137	181	1.55 (0.85-2.82)	-
1 st -4 th grade	52.0 (10.0)	50	60	1.41 (0.72-2.76)	-
4 th -8 th grade	53.7 (9.9)	115	105	1.07 (0.58-1.98)	-
Higher than 9 th grade	53.9 (9.2)	27	23	1 ^r	-
Religion					
Orthodox	50.2 (9.8)	4	10	0.52 (0.13-2.05)	-
Protestant	52.6 (9.7)	286	310	1.22 (0.58-2.55)	-
Catholic	51.6 (11.8)	26	34	1.00 (0.41-2.42)	-
Muslim	50.8 (9.1)	13	17	1 ^r	-
Ethnicity					
Sidama	52.3 (9.9)	320	359	1 ^r	-
Others	52.3 (10.0)	9	12	0.84 (0.35-2.01)	-

1^r set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables and CRP status

Zinc Deficiency, Parity and Birth Interval

On average the serum zinc concentration among women with parity of 0, 1-2, 3-4 and >5 were 54.4 (± 9.8), 53.4 (± 9.3), 51.8 (± 10.1) and 47.1 (± 9.8) $\mu\text{g/dl}$, respectively. Based on ANOVA, the overall difference across the groups was significant and grand multiparas had substantially lower serum zinc concentration than the rest of the groups ($F=10.481$, $P=0.000$). According to the multivariate linear regression model, a unit increment in parity was significantly associated with 0.76 $\mu\text{g/dl}$ decline in serum zinc concentration ($t=3.581$, $p=0.000$). Likewise, compared to nulliparas, the risk of ZD was 1.70 (95% CI: 1.09-3.23) times elevated amongst multiparas.

Among 553 women who had at least a birth in the preceding 5 years of the survey, the pattern of association between birth interval and zinc status was appraised. In general the shorter the interval the lower the serum zinc concentration and the higher the risk of deficiency. A significant net difference of 2.9 $\mu\text{g/dl}$ was observed between women who had short (<24 months) and optimal (≥ 24 months) birth intervals ($t=2.547$, $p=0.013$). The risk of ZD was 1.97 (95% CI: 1.07-4.24) times raised in the former group.

Zinc Deficiency and Dietary Factors

Based on a simple linear regression model, a unit increment in HFIAS was associated with 2.23 $\mu\text{g/dl}$ decline in serum zinc level ($t=14.921$, $p=0.000$). After adjustments were made for other significant variables, a unit increment was associated with 0.93 $\mu\text{g/dl}$ decline in zinc level ($t=7.523$, $p=0.000$). Likewise, women who had above the mean HFIAS score were 5.07 (95% CL: 3.67-6.99) times increased odds of ZD than their counterparts. In addition, a 5.2 $\mu\text{g/dl}$ net difference in mean zinc concentration was observed between the abovementioned two categories and the variation was substantially significant ($t=7.588$, $p=0.000$).

About 451 (64.4%) and 249 (35.6%) of the respondents identified *enset* and cereals (mainly maize) as their staple diets, respectively. On average a net zinc concentration difference of 2.5 $\mu\text{g/dl}$ was observed between the two groups in favor of the *enset* group ($t=3.035$, $P=0.003$). Based on the multivariate linear model, the adjusted difference was even moved up to 3.60 $\mu\text{g/dl}$ ($t=6.971$, $P=0.000$). The risk of ZD was 2.25 (95% CI: 1.61-3.16) times higher in maize staple diet group.

Overall the mean DDS was 4.3 (± 1.7) and about 243 (34.7%), 318 (45.4%) and 139 (19.9%) of the subjects had low, optimal and high DD, respectively. Based on the dietary intake in the preceding day of the survey, the most frequently consumed food types were root and tubers (78.4%), cereals (52.7%) and legumes (52.2%). Using multivariate linear regression model, a unit increment in DDS was associated with a 2.54 $\mu\text{g/dl}$ rise in serum zinc level ($t=16.757$, $P=0.000$). Compared to women with high DD, the risk of ZD was 1.87 (95% CI: 1.02-2.91) and 2.57 (95% CI: 1.57-4.18) times elevated in women with medium and low DD, consecutively.

About a quarter, 181 (25.9%), of the subjects consumed animal source foods in the preceding day of the survey. On average, the mean zinc concentration was elevated by 4.7 $\mu\text{g/dl}$ among women who reported consumption of animal based foods ($t=6.088$, $P=0.000$). In the multivariate linear model, the gap was reduced to a significant 0.98 $\mu\text{g/dl}$ difference ($t=2.007$, $P=0.039$). Similarly, using the logistic model, consumption of animal based foods in the reference period was associated to a reduced risk of ZD with OR of 2.51 (95% CI: 1.70-3.72).

About two-third, 221 (31.6%), of the pregnant women were anemic. Anemia and ZD frequently occurred together. Nearly one-fifth, 149 (21.3%), of the subjects had both and amongst anemic women, 149 (67.4%) had ZD. Similarly, among zinc deficient women 149 (40.3%) were anemic. After controlling other significant variables and CRP status, anemia and ZD were associated with OR of 2.37 (95% CI: 1.96-3.33).

In order to appraise the relationship between frequency of coffee intake and zinc status, respondents were asked to estimate the volume (per 70 ml cup) of coffee intake in a typical day. On average every woman takes 3.3 (± 1.4) cups of coffee/day. Using simple and multiple linear models, every cup of coffee intake was linked with 0.77 ($t=2.939$, $P=0.003$) and 0.53 $\mu\text{g/dl}$ ($t=3.917$, $P=0.000$) decline in zinc concentrations. The zinc level was also compared between respondents who had below and above average frequency of coffee intake. On average a significant 1.5 $\mu\text{g/dl}$ difference was observed between the groups ($t=2.062$, $P=0.040$). Based on the logistic model, women with more than average intake had 1.41 (95% CI: 1.06-1.84) times increased risk.

Zinc Deficiency and Prenatal Iron Supplementation

About 140 (20.0%) of the respondents took iron supplements (daily dose of 150 mg ferrous sulphate and 500 µg folate) at least once in the preceding 28 days of the survey. Nevertheless during the reference period only 105 (15.0%) reported full compliance. So as to evaluate the effect of prenatal iron supplementation on zinc status, the zinc concentration was compared between women who didn't take any supplement (n=560) and women who reported full compliance (n=105) in the preceding four weeks of the survey. The zinc levels for the two groups were 52.9 (±9.8) and 50.9 (±10.0) µg/dl, respectively. The difference was marginally insignificant ($t=1.966$, $P=0.051$). According to the logistic model, the risk of ZD in iron supplemented group was not significantly elevated with OR of 1.34 (95% CI: 0.90-2.13).

The relationship between the duration of prenatal iron supplementation and zinc status was also explored. The serum zinc levels for those who have been on the supplement for less than 4 weeks, 4-8 weeks and more than 8 weeks were 50.4 (±9.7), 52.9 (±10.2) and 49.6 (±10.2) µg/dl, respectively. The overall difference was not statistically significant ($F=0.907$, $P=0.406$).

Zinc Deficiency and Maternal Workload

In order to evaluate the relationship between maternal workload and zinc status, the most common and laborious activities in the community were identified and their frequency in the preceding week of the survey were quantified. Using PCA, the level of workload was summarized into an index of five ordinal categories namely lowest, lower, middle, higher, and highest. The mean zinc in µg/dl for the categories were 51.9 (±9.9), 53.1 (±10.4), 52.5 (±9.0), 51.6 (±10.7) and 52.7 (±10.0), respectively. No peculiar pattern was observed across the categories and the differences were not significant ($F=0.509$, $P=0.729$). Similarly the risk of ZD was comparable across the categories.

Zinc Deficiency and Health Care Related Factors

The serum zinc level and the prevalence of ZD were compared across different variables related to health care. The intension was to see how potent the factors are in terms of reducing the burden of ZD. However, based on both the linear and logistic models, distance from nearby health facility, frequency of ANC, and nutrition education during pregnancy were not significantly associated with the dependent variable (Table 7).

Table 7: Reproductive, dietary and health care related correlates of zinc deficiency among pregnant women in rural Sidama, Feb 2001.

Variables (n=700)	Serum zinc $\mu\text{g/dl}$ mean(sd)	Frequency		Crude OR (95% CI)	Adjusted OR (95% CI) **
		ZD+	ZD-		
Parity					
0	54.4 (9.8)	52	64	1 ^r	1 ^r
1-2	53.4 (9.3)	138	138	1.23 (0.80-1.90)	0.72 (0.46-1.15)
3-4	51.8 (10.1)	126	105	1.48 (0.94-2.31)	0.77 (0.48-1.24)
≥ 5	47.1 (9.8)	55	22	3.08 (1.66-5.69)*	1.70 (1.09-3.23)*
Staple diet					
Enset based	53.3 (9.0)	217	234	1 ^r	1 ^r
Cereal based	50.8 (10.5)	154	95	1.74 (1.28-2.40)*	2.25 (1.61-3.16)*
Diet Diversity					
Low	50.3 (9.6)	162	81	4.78 (3.04-7.51)*	2.57 (1.57-4.18)*
Medium	52.0 (9.9)	168	150	2.68 (1.75-4.10)*	1.87 (1.02-2.91)*
High	56.9 (9.0)	41	98	1 ^r	1 ^r
Ate animal source foods in the previous day					
No	51.2 (9.1)	312	207	3.12 (2.18-4.45)*	2.51 (1.70-3.72)*
Yes	55.9 (9.9)	59	122	1 ^r	1 ^r
HFIAS					
Below the mean value	56.4 (8.7)	116	241	1 ^r	1 ^r
Above the mean value	51.2 (9.4)	255	88	6.02 (4.33-7.36)*	5.07 (3.67-6.99)*
Frequency of coffee intake/day					
≤ 3 coffee cups	53.1 (9.8)	189	141	1 ^r	1 ^r
> 3 coffee cups	51.6 (10.0)	182	188	1.38 (1.02-1.89)*	1.41 (1.06-1.84)*
Maternal workload					
Lowest	51.9 (9.9)	76	67	1 ^r	-
Lower	53.1 (10.4)	69	72	1.12 (0.67-1.79)	-
Middle	52.5 (9.9)	75	63	0.94 (0.59-1.51)	-
Higher	51.6 (10.7)	82	59	1.17 (0.73-1.88)	-
Highest	52.7 (10.0)	69	68	1.37 (0.85-2.20)	-
One-way walking distance from nearby health facility					
0-30 minutes	52.5 (9.9)	311	287	0.76 (0.50-1.16)	-
Longer than 30 minutes	51.6 (10.2)	60	42	1 ^r	-
Frequency of ANC					
0	53.4 (9.9)	184	180	1 ^r	-
1-2	51.3 (9.8)	171	136	1.23 (0.48-2.20)	-
≥ 3	50.6 (9.5)	16	13	1.20 (0.56-2.58)	-
Received nutrition education during the pregnancy					
Yes	51.4 (10.2)	99	76	1.21 (0.86-1.71)	-
No	52.7 (9.8)	272	253	1 ^r	-

1^r set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables and CRP status

Correlates of Prenatal Vitamin A Status

Vitamin A Deficiency and Socio-economic Factors

Bivariate correlation analysis showed a moderate but significant relationship between maternal age and serum retinol concentration ($r=-0.324$, $P=0.000$). Based on a multivariate linear model, a unit increment in maternal age (in years), was associated with 0.01 $\mu\text{mol/l}$ decline in retinol concentration ($t=2.474$, $P=0.014$). Overall, the retinol level declined from 0.89 (± 0.44) $\mu\text{mol/l}$ in women aged 15-24 years to 0.85 (± 0.40) and 0.71 (± 0.41) in women aged 25-34 and 35-49 years, respectively. The overall difference was significant ($F=6.89$, $P=0.001$). Compared to the youngest group, the risk of VAD was 2.23 (95% CI: 1.31-3.81) times elevated among women in the oldest age group.

Educated women had superior vitamin A status and substantially reduced risk of VAD. The retinol concentration in illiterates was 0.80 (± 0.43) $\mu\text{mol/l}$, whereas the corresponding value for those who completed 1st-4th grade and beyond 4th grade education were 0.87 (± 0.38), 0.93 (± 0.38) $\mu\text{mol/l}$, respectively ($F=19.682$, $P=0.000$). This corresponds to 14.0% reduction in retinol level among illiterates compared to women educated beyond 4th grade. The multivariate logistic model showed that illiterates had 1.73 (95% CI: 1.02-2.92) times increased risk of VAD compared to those educated beyond 4th grade.

Based on ANOVA and logistic regression analysis, household wealth index was not found to be a significant correlate of VAD. Nevertheless, women's involvement in IGAs illustrated affirmative influence. Retinol level among women who were enrolled in IGA was significantly higher than their counterparts ($t=2.557$, $P=0.012$) and the risk of VAD was 2.18 (95% CI: 1.29-3.69) times elevated amongst women devoid of self income.

Bivariate correlation analysis illustrated no significant association between serum retinol concentration and altitude ($r=-0.059$, $P=0.119$). The serum retinol showed inclination to rise as we move from the lowlands to the highlands. However the difference was not statistically significant ($F=1.138$, $P=0.251$). Likewise, the risk of VAD was not significantly different across the three agro-ecological zones.

Vitamin A Deficiency and Dietary Factors

Alike zinc deficiency, women who reported higher DD in the previous day of the survey had better vitamin A status and reduced risk of VAD. In the correlation analysis a significant positive association was observed between the two variables ($r=0.330$, $P=0.000$). Using a multivariate linear model where 26.2% of the variability in serum retinol level was explained, a unit rise of DD was related with 0.04 $\mu\text{mol/l}$ increment in serum retinol concentration ($t=4.429$, $P=0.000$). Similarly the risk of VAD was 1.94 (95% CI: 1.17-3.19) times elevated among women with low DD compared to those with high DD.

Likewise women who consumed animal source foods in the preceding day of the survey had reduced burden of VAD. Based on independent t-test analysis, on average a significant 0.10 $\mu\text{mol/l}$ difference was observed between women who ate animal source foods in the reference period and their counterparts ($t=2.804$, $P=0.004$). After adjustments were made for other important variables, the net difference was found to be 0.12 $\mu\text{mol/l}$ ($t=3.575$, $P=0.000$). The logistic regression model showed 1.51 (95% CI: 1.04-2.13) times increased risk of VAD among women who did not consume animal based foods in the reference period.

Bivariate correlation analysis showed a weak but significant association between MUAC and serum retinol concentration ($r=0.217$, $P=0.000$). Using multivariate linear regression model where other significant variables were controlled, the relationship remained significant with regression coefficient of 0.02 ($t=2.515$, $P=0.012$). Nevertheless the logistic model did not witness parallel association. The risk of VAD was not significantly elevated among women with SAM (MUAC<220 mm) with AOR of 1.26 (95% CI: 0.88-1.81).

Serum retinol and zinc concentrations were significantly and positively correlated ($r=0.322$, $P=0.000$). Using multivariate linear regression model where other significant variables were controlled, a $\mu\text{mol/l}$ increment in zinc concentration was associated with a significant 0.03 $\mu\text{mol/l}$ rise in retinol level ($t=3.545$, $P=0.000$). Categorical data analysis also indicated that the two deficiencies tend to occur together. A quarter, 24.9% (95% CI: 21.7-28.1%), of the subjects had concomitant VAD and ZD. Further about two-third, 65.6% (95% CI: 59.9-71.4%), of VA deficient subjects had ZD (Figure 6). After controlling potential joint determinants, VAD and ZD were significantly associated to each other with AOR of 1.80 (95% CI: 1.28-2.53).

Vitamin A Deficiency and Reproductive Factors

Serum retinol steadily declined from 0.86 (± 0.40) $\mu\text{mol/l}$ in nulliparas to 0.85 (± 0.41), 0.84 (± 0.42) and 0.75 (± 0.40) $\mu\text{mol/l}$ in parity categories of 1-2, 3-4 and ≥ 5 , respectively. The global difference was significant ($F=2.886$, $P=0.035$). Using the linear regression model, parity and retinol concentration were negatively associated with adjusted coefficient of 0.036 ($t=3.343$, $p=0.001$). The logistic model also indicated that multiparas had 2.25 (95% CI: 1.20-4.22) times increased risk of VAD than nulliparas. On the other hand, retinol levels in women with short (< 24 months) and optimal (≥ 24 months) birth intervals were 0.72 (± 0.45) and 0.84 (± 0.41) $\mu\text{mol/l}$, respectively. The difference was marginally significant ($t=2.032$, $P=0.043$). The risk of VAD was 2.11 (95% CI: 1.15-3.86) times higher in the earlier group.

Vitamin A Deficiency and Health Care Related Factors

The risk of VAD was compared across various health care service related factors. Women who had to walk over 30 minutes to access the nearby health facility did not have significantly increased risk of VAD with OR of 0.88 (95% CI: 0.57-1.36). The risk of VAD was not elevated among pregnant women who did not get nutrition education during the current pregnancy with OR of 0.89 (95% CI: 0.62-1.26). Likewise the risk of VAD was comparable across various categories of frequency of ANC during the index pregnancy (Table 8).

Serum Retinol and CRP Status

Alike zinc, serum retinol found to have negative association with elevated CRP. The retinol levels amongst CRP negative and positive subjects were 0.85 (± 0.42) and 0.66 (± 0.34) $\mu\text{mol/l}$, respectively. Based on independent t-test, the difference was significant ($t=3.828$, $p=0.000$) and the observed unadjusted net difference was equivalent to 22.5% reduction in serum retinol concentration in CRP positive subjects. According to the multivariate linear regression model, elevated CRP was associated with a substantial 0.13 $\mu\text{mol/l}$ decline in retinol level. Overall, elevated CRP was significantly associated with low serum retinol level with AOR of 2.21 (95% CI: 1.22-4.02).

Table 8: Association between vitamin A status and various potential risk factors among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011.

Variables	Retinol $\mu\text{mol/l}$ mean (sd)	Frequency		Crude OR (95% CI)	Adjusted OR (95% CI) **
		VAD+	VAD-		
Staple diet					
Enset based	0.81 (0.41)	180	271	1.28 (0.93-1.77)	-
Cereal based	0.88 (0.42)	85	164	1 ^r	-
Dietary diversity					
Low	0.75 (0.41)	119	124	2.55 (1.63-4.00)*	1.94 (1.17-3.19)*
Medium	0.87 (0.41)	108	210	1.37 (0.88-2.12)	1.10 (0.70-1.72)
High	0.91 (0.43)	38	101	1 ^r	1 ^r
Consumed animal source foods in the previous day					
No	0.81 (0.42)	216	303	1.92 (1.33-2.78)*	1.51 (1.04-2.13)*
Yes	0.91 (0.39)	49	132	1 ^r	1 ^r
SAM					
No	0.87 (0.42)	178	330	1 ^r	1 ^r
Yes	0.75 (0.39)	87	105	1.54 (1.10-2.15)*	1.26 (0.88-1.81)
Zinc status					
Non deficient	0.92 (0.41)	91	238	1 ^r	1 ^r
Deficient	0.76 (0.40)	174	197	2.31 (1.68-3.17)*	1.80 (1.28-2.53)*
Parity					
0	0.89 (0.40)	34	82	1 ^r	1 ^r
1-2	0.85 (0.41)	102	174	1.41 (0.86-2.26)	1.14 (0.69-1.87)
3-4	0.84 (0.42)	91	140	1.57 (0.97-2.53)	1.35 (0.81-2.26)
≥ 5	0.72 (0.40)	38	39	2.35 (1.29-4.28)*	1.92 (1.02-3.64)*
Two-way walking distance to the nearby health facility					
0-30 minutes	0.83 (0.41)	229	369	1 ^r	-
> 30 minutes	0.89 (0.44)	36	66	0.88 (0.57-1.36)	-
ANC follow up					
0	0.87 (0.41)	121	243	1 ^r	1 ^r
1-2	0.80 (0.43)	134	173	1.56 (1.14-2.13)*	1.42 (0.97-2.02)
≥ 3	0.86 (0.41)	10	19	1.06 (0.48-2.34)	0.70 (0.30-1.64)
Nutrition education during pregnancy					
Yes	0.80 (0.41)	70	105	1 ^r	-
No	0.85 (0.42)	195	330	0.89 (0.62-1.26)	-

1^r set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables

Prevalence and Determinants of LBW

In the cohort study 650 eligible pregnant women were followed and 575 (88.5%) qualified to the ultimate analysis on birthweight. The exclusions were due to loss to follow-up, weighed later than 72 hours of birth, fetal loss, multiple birth and early neonatal death (Figure 3). The retained (n=575) and excluded (n=125) subjects were not statistically different in wealth index ($P=0.898$), agro-ecological zone ($P=0.284$), literacy ($P=0.802$), CRP status ($P=0.854$), maternal height ($P=0.372$), maternal MUAC ($P=0.763$), prenatal serum zinc ($P=0.056$) and retinol ($P=0.219$) concentrations.

The overall mean birthweight was 2896 (± 423) g. About 16.5% (95 % CI: 13.5-19.6%) of the babies were born with LBW. The detailed information on the risk factors of LBW is given in Table 1 of paper III. Among the socio-demographic factors, maternal education was significantly associated to the birthweight of the offspring. On average the weight of babies born to literates was significantly higher by 192 g ($t=5.276$, $P=0.000$) and the risk of LBW was 1.80 (95% CI: 1.11-2.93) times elevated among illiterates. Neither household wealth index nor women's involvement in IGA was a significant predictor of LBW.

Based on simple linear regression analysis, one year increment in maternal age was significantly associated with 7g rise in birthweight ($t=2.243$, $P=0.025$). However in the multivariate linear regression model which explained 20.9% of the variability of birthweight, the association turned out to be insignificant ($t=1.091$, $P=0.276$). Likewise, the risk of LBW was comparable across categories of maternal age. Incidence of LBW tended to be higher in primipara women. On average babies born to parous women were 129 g heavier than those born to primis ($t=2.481$, $P=0.013$) and the risk of LBW was 1.16 (95% CI: 1.02-1.35) times higher among primiparas.

Birthweight was positively and significantly influenced by male sex of the offspring. Independent t-test analysis indicated that male babies weigh significantly heavier than females by 103g ($t=2.931$, $P=0.004$). Accordingly to the multivariate linear model, a 102 g difference were observed between the two sexes ($t=3.194$, $P=0.001$). Overall female babies had 1.16 (95% CI: 1.02-1.35) times elevated risk of LBW.

The weight of babies born to women who had elevated CRP during pregnancy (2748 ± 429 g) was significantly lower than their counterparts (2910 ± 429 g) ($t=2.601$, $P= 0.010$) and according to the multivariate linear model elevated CRP was associated with a significant 180 g reduction in birthweight ($t=2.830$, $P= 0.005$). Yet the log-binomial model failed to witness any significant association between LBW and CRP status.

Maternal stunting and thinness turned out to be strong predictors of birthweight. The weight of babies born to thin women (2697 ± 361 g) was significantly lower compared to their counterparts (2971 ± 361 g) ($t=7.255$, $P= 0.000$). Likewise a 195 g (95% CI: 95-294 g) difference was observed between babies born to non-stunted and stunted women. According to the linear model, a cm increment in maternal height and MUAC were associated with 11 g ($t=4.458$, $P= 0.000$) and 71 g ($t=7.941$, $P= 0.000$) increases in birthweight. Overall the risk of LBW was 1.63 (95% CI: 1.06-2.51) and 1.52 (95% CI: 1.03-2.25) times elevated in stunted and thin women.

The risk of LBW did not significantly differ across categories of agro-ecological zone, ANC, type of staple diet and distance from the nearby health facility (Table 1 of paper III).

Prenatal Zinc and Vitamin A as Predictors of Birthweight

Bivariate correlation analyses witnessed very weak but positive associations between prenatal serum zinc and birthweight ($r=0.089$, $P= 0.032$) and serum retinol and birthweight ($r=0.092$, $P= 0.027$). Likewise using independent t-test analysis, statistically significant 82g (95% CI: 9-150g) and 76g (95% CI: 6-145g) decrease on birthweight was observed in babies born to women who had prenatal VA and zinc deficiencies, respectively. In the bivariate log-binomial model, the deficiencies were also marginally associated to LBW.

However in the multivariate linear model adjusted for parity, maternal age, CRP status, MUAC and maternal height, serum zinc ($\beta=1.6$, $t=0.977$, $P= 0.329$) and retinol ($\beta=-0.1$, $t=0.086$, $P= 0.932$) concentrations were not significantly associated to birthweight. Likewise, in the multivariate log binomial model where adjustments were made for potential confounders, VAD and ZD were not significantly associated to LBW with adjusted RR of 1.25 (95 CI: 0.86-1.82) and 1.27 (95% CI: 0.86-1.87), respectively.

The log-binomial model was separately developed for women who were at their second and third trimesters during the time of exposure assessment so as to evaluate the possibility of trimester specific effects of the deficiencies under consideration. The occurrence of ZD in the second or third trimester was not associated to LBW with RR of 1.31 (95 % CI: 0.73-2.38) and 0.81 (95% CI: 0.46-1.40), respectively. Similarly VAD that occurred in the second or third trimester was not linked with LBW with RR of 1.26 (95% CI: 0.77-2.07) and 1.35 (95% CI: 0.71-2.57), respectively.

In order to evaluate the synergetic effects of prenatal zinc and VA deficiencies in causing LBW, the study subjects were classified into four groups based on VA and zinc deficiency statuses and SI was calculated accordingly (Table 9). VAD and ZD did not show any interaction in causing LBW with SI of 1.04 (95% CI: 0.17-6.28).

Table 9: Prenatal zinc and retinol status as predictors of LBW in rural Sidama, Southern Ethiopia, Jan-Oct 2011.

Variables	Birthweight		Crude RR	Adjusted RR
	Low	Normal		
Vitamin A status				
Normal	48	299	1 ^r	1 ^r
Deficient	47	181	1.49 (1.03-2.15)*	1.25 (0.86-1.82)
Zinc status				
Normal	34	225	1 ^r	1 ^r
Deficient	61	255	1.47 (1.01-2.16)*	1.27 (0.86-1.87)
Zinc-VA status				
Normal zinc and Normal VA	20	155	1 ^r	1 ^r
Zinc deficient and VA normal	28	144	1.42 (0.84-2.43)	1.30 (0.72-2.31)
VA deficient and zinc normal	14	70	1.46 (0.78-2.74)	1.31 (0.66-2.61)
Zinc deficient and VA deficient	33	111	2.01 (1.20-3.34)*	1.76 (1.00-3.11)

Effect of Prenatal Zinc Supplementation on Birthweight

In order to assess the effect of prenatal zinc supplementation on birthweight a meta-analysis of RCT was conducted. Seventeen studies were included based on the predefined inclusion criteria irrespective of place and time of the study. The details on the information extracted from the specific studies are available in Table 1 of paper IV.

The analysis included four studies from the USA, three from the UK, six from Asian countries, three from Latin American countries and a study in Africa. The studies involved 6,209 pregnant women and the number of study subjects ranged from 1,075 in Nepal (32) to 52 in the UK. Six of the studies were community based whereas the remaining were health institution based. In 12 of the studies the dose of the supplement was between 15-25 mg/day whereas in the remaining studies higher doses were supplemented.

The Effect Size (ES) estimates of the specific studies illustrated that only three studies reported positive association between zinc supplementation and birthweight. Thirteen of the studies found no association and the remaining one study reported negative association. Based on fixed and random effect models, the pooled effect sizes were 0.0268 (95% CI: 0.0764 to -0.0229) and 0.0712 (95% CI: 0.1619 to -0.0194) respectively. Accordingly, zinc supplementation did not show any significant effect on birthweight (Figure 7).

Table 10: Mean effect size in different categories of RCTs on the association between prenatal zinc supplementation and birthweight, 1984-2009.

Stratifying variable	Total sample size	ES with 95% CI (Fixed effect model)	ES with 95% CI (Random effect model)
Type of country			
Developed	1863	0.107 (0.197 to -0.017)	0.107 (0.199 to -0.016)
Developing	4345	-0.008 (0.051 to -0.068)	0.057 (0.183 to -0.070)
Type of study			
Community based	3476	0.101 (0.174 to 0.026)	0.129 (0.267 to -0.010)
Health institution based	2732	-0.031 (0.041 to -0.103)	0.075 (0.268 to -0.118)
Dose of supplementation			
15-25 mg/day	4869	0.016 (0.072 to -0.040)	0.040 (0.123 to -0.043)
26-62 mg/day	1339	0.067 (0.175 to -0.041)	0.169 (0.457 to -0.119)

To control the possible effect of confounders, stratification was made based on type of study country (developed or developing), dose of supplementation (optimal or high dose), and type of study (health institution-based or community-based). Nevertheless prenatal zinc supplementation was not associated with birthweight irrespective of the aforementioned factors (Table 10).

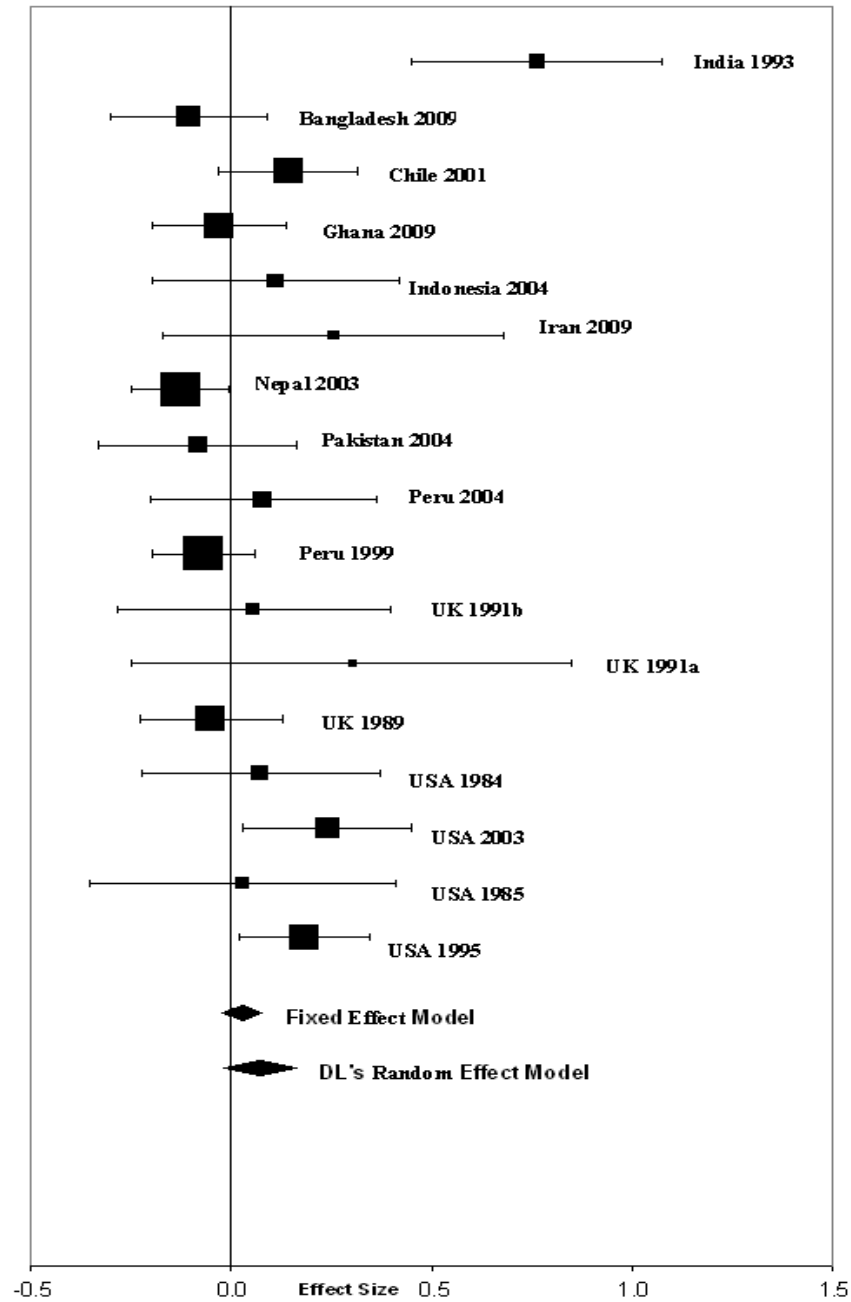


Figure 7: Forest plot of 17 randomized controlled trials on the association between prenatal zinc supplementation and birthweight, 1984-2009.

Summary of the Core Results of the Papers Included in the Dissertation

Table 11: Summary of the core results of the papers included in the dissertation.

No	Title of the paper	Main findings
1	Prevalence of prenatal zinc deficiency and its association with socio-demographic, dietary and health care related factors in Rural Sidama, Southern Ethiopia: A cross-sectional study	<ul style="list-style-type: none"> • About 53.0% of the subjects were zinc deficient. • The majority of the explained variability of serum zinc was due to dietary factors like household food insecurity level and DDS. • Maternal literacy and involvement in IGA showed affirmative influence on zinc status. • The risk of ZD was higher among women who were reliant on cereals as their staple diet. • Increased parity and maternal age were negatively associated with prenatal zinc status. • Anemia and ZD tend to occur together. About a fifth of the subjects had both deficiencies. • Frequency of coffee intake was negatively association to serum zinc level. • Prenatal iron supplementation did not increase the risk of ZD.
2	Prevalence and correlates of prenatal Vitamin A deficiency in rural Sidama, Southern Ethiopia.	<ul style="list-style-type: none"> • The prevalence of VAD was 37.9%, and an additional 28.7% of the subjects had marginal status. • The risk of VAD was higher among illiterates and women devoid of self income. • Advanced gestational age and elevated CRP were negatively associated with VA status. • Advanced parity and maternal age were also negative correlates of VA status. • Women with low DDS were more likely to be VA deficient. • VAD and ZD tend to occur together. About a quarter of the subjects had both of the deficiencies.
3	Independent and joint effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama, Southern Ethiopia: Community based prospective cohort study.	<ul style="list-style-type: none"> • The mean birthweight was 2896 g and about 16.5% of the babies had LBW. • Prenatal ZD and VAD were not significantly associated to LBW. • The occurrences of ZD and VAD, neither in the 2nd nor 3rd trimester, were associated to LBW. • VAD and ZD did not show synergetic interaction in causing LBW. • Risk factors of LBW were maternal illiteracy, female sex of the newborn, primiparity, elevated CRP and maternal thinness and stunting.
4	Systematic review on the effect of prenatal zinc supplementation on birthweight: Meta-analysis of 17 RCTs.	<ul style="list-style-type: none"> • Prenatal zinc supplementation does not improve birthweight.

VI. Discussion

The study estimated the prevalences of ZD, VAD and ID based on cross-sectional data. Consequently the reported figures are liable to seasonality bias. The baseline study was carried out in January and February which are neither food insecure nor food surplus months in the area. Higher prevalence would have been anticipated if the study had been conducted in food insecure months. On the other hand, the study might have overestimated the prevalence figures as women in the first trimester were largely underrepresented. In the first trimester, as the degree of hemodilution is less pronounced, the burden of the deficiencies is expected to be lower.

According to IZiNCG, the risk of ZD is considered to be elevated and of public health concern when its prevalence exceeds 20% (71). Similarly, with reference to the cutoff points of WHO (16), the public health significance of VAD is considered to be of severe degree when its prevalence surpasses 20%. Hence the reported prevalence figures of ZD (53%) and VAD (38%) are indicative of the substantial public health connotation of the deficiencies in the locality.

So far a study in Ethiopia attempted to estimate the prevalence of prenatal ZD. The study was also conducted in Sidama zone (6, 70). Nonetheless, it had limited geographical coverage, smaller sample size and applied convenience sampling technique. According to it, the prevalence of ZD was 72% and the mean plasma zinc level was 45.6 $\mu\text{gm/dl}$ (6). Compared to the current study, it reported higher prevalence of ZD. The discrepancy can be explained by the time gap and seasonal differences between the studies. The previous study might also have overestimated the problem as it only included pregnant women in the third trimester.

In 2004, based on national food supply statistics, IZiNCG estimated that 21.1% of Ethiopian population was at risk of inadequate dietary zinc intake (60). Accordingly, the country was classified in medium-risk category for the deficiency. Though the current study was conducted in an area that has its own peculiar dietary attribute, our measured prevalence of ZD (53%) immensely exceeds the IZiNCG national estimate.

So far in the developing world number of studies attempted to estimate the prevalence of prenatal ZD. However it is difficult to compare the figures as various cutoff points were applied to define ZD. Studies in India (63) and Malawi (68) reported prevalences of 65% and 36% using lower cut-off points of 66 and 49.5 µg/dl, respectively. A study in Iran got 49.0% prevalence after using 70 µg/dl as cutoff point (65). Studies conducted in Indonesia (66) and Nigeria (69) reported 21.8% and 45.8% prevalence figures respectively without stating their cutoff points.

So far three studies in Ethiopia assessed the prevalence of subclinical prenatal VAD. A study conducted among 85 pregnant women in Sidama zone witnessed 27% prevalence (18). The current study estimate of 38% might be due to the fact that the earlier study was conducted among conveniently selected women in relatively accessible localities. The other two studies were carried out among ANC attendants in Gondar hospital, Northwestern Ethiopia. The studies reported 17.2% (17) and 18.4% (19) prevalences. Compared to the current study, the studies reported lower prevalences perhaps due to the reason that they were conducted in an urban setup where the extent of the problem is expected to be lower.

The study revealed the elevated burden of multiple micronutrients deficiency during pregnancy. Based on the prevalence of iron, VA and zinc deficiencies, approximately one-third, of the subjects had two or more concomitant deficiencies and 5% had all of the three deficiencies. A couple of studies conducted in Asia empirically witnessed the simultaneous occurrence of micronutrient deficiencies. A study in Nepal measured 11 micronutrients and found 20% prevalence of two or more deficiencies and 18% prevalence of five or more deficiencies (263). A study in India considered 6 deficiencies and concluded that approximately 55% of the subjects had concomitant iron and zinc deficiencies (264).

The findings of the linear regression model showed that 53.5% of variability of serum zinc level was explained by ten variables. The unexplained 46.5% of the variability might be attributable to range of variables (like serum albumin, various illnesses, intestinal and hemoparasites etc) which were not included in the study. Removing dietary intake related variables from the model would reduce the r-squared value to 15.2%. This is strong evidence that non-dietary factors like maternal age, parity, gestational age have significant but relatively less importance in explaining serum zinc concentrations.

Based on the data of 61 pregnant women included in US National Health and Nutrition Examination Survey (NHANES), IZiNCG suggested trimester specific cutoffs points for defining prenatal ZD (60). The suggested point for the first trimester was 56 $\mu\text{g}/\text{dl}$. However, despite the distinct trend for declining serum zinc concentration throughout pregnancy, the cutoff point for the second and third trimesters didn't differ significantly; accordingly, a pooled cutoff point of 50 $\mu\text{g}/\text{dl}$ was recommended (60). Nonetheless in the current study an approximately linear decline in zinc level was witnessed throughout pregnancy. In addition, parallel to studies conducted elsewhere (65, 265), the zinc level in the last trimester was significantly lower than that of the second trimester. The finding is also consistent with the understanding that hemodilution extends well into the third trimester (36, 68). This may indicate the need of having different cutoff points for defining ZD during the second and third trimesters.

According to IZiNCG serum zinc concentration exhibits diurnal variation and fluctuates as a result of physiologic reaction to acute inflammation/infection and fasting (60). However, in the current study fasting status was not associated to serum zinc level. Though significant disparity of 3 $\mu\text{g}/\text{dl}$ was observed between samples collected in the morning and afternoon, the discrepancy was not as large as the 6-12 $\mu\text{g}/\text{dl}$ difference observed in NHANES (60). Likewise the difference observed between CRP positive and negative samples was not as large as expected (266). The finding suggests that in predominantly zinc deficient community the effect of the aforementioned factors may not be as substantial as expected in affluent community.

Intuitively superior household economic standing enhances zinc status. Previous studies in Nigeria (69), India (75) and Indonesia (66) noted the same. However, in the current study household wealth index was not associated to maternal zinc status. Nevertheless involvement of women in IGAs found to have positive influence on prenatal zinc level, irrespective of maternal educational status. This might be due to the reason that in Ethiopia maternal income is usually directly spent to cover household food expenditures. The fact that household wealth status was measured based on a relative rather than an absolute scale may also explain the unanticipated result. Such kind of measurement might not be very potent in differentiating wealth groups when the study population is fairly homogenous in wealth status.

The study witnessed a negative association between maternal age and zinc status. The association could not be explained by the expected extended parity in older age groups as maternal age was statistically controlled. The finding is parallel to the understanding that serum zinc level reaches peak during adolescence and young adulthood, and then declines (51). Previous studies conducted elsewhere reported comparable findings. A study in Nigeria found consistent increment in prevalence ZD as maternal age advances (69). A study in Iran reported higher prevalence of ZD in women older than 30 years compared to those aged 20-30 years (65).

The study witnessed the deleterious effect of grand multiparity and close birth intervals on maternal zinc status. The finding is in confirmation of the understanding that repeated pregnancies exhaust maternal nutrient stores. Earlier studies also concluded likewise. A study in rural Malawi reported that frequent reproductive cycling was inversely associated with hair zinc concentrations (68). Similarly, in Nigeria a negative relation between zinc status and parity was noted (69). A study amongst low income pregnant women in USA reported lower mean plasma zinc in multiparous compared to primiparous women (76). However, two studies in Iran (65, 74) and a study in South Africa (84) did not show any significant association. Such conflicting findings might be explained by the potential variation in dietary zinc intake across the study communities. In places where dietary intake is optimal, depleted maternal stores can be replenished swiftly; hence the negative effect of extended parity might not be apparent.

The zinc status of women from *enset* staple diet category was significantly better than women from maize staple diet category. The association couldn't be explained by an underlying variation in dietary diversity and food security as they had been statistically controlled. A previous study among pregnant women in the locality pointed out that mean zinc intakes from the maize and *enset*-based diets were 6.3 and 3.7 mg/day, respectively; however, the bioavailability zinc in *enset* based diets was twice than that of maize based diets (phytate to zinc molar ratio of 21.1 and 10.8 respectively) (18). Nevertheless, the study did not detect statistically significant difference in serum zinc values (6.86 and 7.05 $\mu\text{mol/l}$ for women from maize and *enset* staple diet areas, respectively).

ZD and anemia were significantly associated. The association cannot be entirely attributed to the joint risk factors of the problems as such factors had been statistically controlled. An earlier study among pregnant women in the locality also identified plasma zinc as the strongest predictor of hemoglobin compared to other significant covariates including ferritin (18). A study in Sudan also reported that among zinc deficient pregnant women the odds of anemia was 2.6 times elevated (83). Parallel findings have also been reported in Iran (74) and Nepal (62). As the studies are cross-sectional it is not viable to implicate causal inference. Nevertheless the etiological role of zinc in causing anemia has to be investigated further. Previous studies have hypothesized that ZD can induce anemia through decreasing erythropoietin and erythroid precursors and shortening the lifespan erythrocytes (267).

On the basis of the hypothesis that iron intake may hamper the absorption of zinc, many community based studies attempted to assess the relation between prenatal iron supplementation and zinc status. A study in Iran reported that pregnant women who were supplemented for iron were more likely to develop ZD (65). Similarly in India among apparently health subjects zinc status indicators were negatively associated with iron intake (75). Nevertheless in the current study, parallel to studies conducted in Nigeria (79), Iran (74, 80) and UK (81, 82), daily iron supplementation was not associated to maternal zinc status. This might be due to the reason that in the study community the intake of iron and zinc rich foods was low; hence, competitive absorption which is expected to occur at higher concentrations might not have taken place.

The study found a negative association between frequency of coffee intake and maternal zinc status. Coffee is known to contain tannin which can potentially inhibit zinc absorption (268). However, empirical evidences on the effect of zinc absorption are lacking. Few available animal model studies reported conflicting conclusions. According to Afsana et al, in rats tannin and related compounds inhibit the absorption of iron but not zinc, copper and manganese (269). According to Aldrian et al, in non-pregnant rats there were no significant effects of coffee on tissue concentrations of zinc, but pregnant rats given coffee had low placenta zinc concentrations compared to controls (270).

Existing standards does not define prenatal VAD based on trimester specific cutoff points. However in the current study gestational trimester and VA status were inversely associated apparently due to the effect of hemodilution. Parallel findings were reported in Zimbabwe (148), India (271) and Turkey (272). In the current study, serum retinol significantly declined by about 22% from the first trimester to third trimester. A study in Indonesia also reported a comparable 20% reduction over the three trimesters (273). In Bangladesh a more substantial 23% reduction in retinol concentration was observed only in the final two trimesters (151). The findings might suggest the need of having trimester specific cutoff points for defining prenatal VAD.

In the current study elevated CRP was associated with 22.5% reduction in serum retinol level. Previous studies also noted the acute phase reactant nature of retinol (51, 274). A study in US concluded that serum retinol and CRP were negatively associated in all males and females older than 30 years (142). A study among Nepalese pregnant women reported that elevated CRP had negative effect not only on retinol but also on serum β -carotene (275). A meta-analysis also linked elevated CRP with 25% decline in retinol level (276). The findings indicate that acute phase response can be a cause for misclassification of VA status. However in the current study it might not possible to exclude a genuine vicious causal association between infection and VAD.

The study witnessed an inverse association between maternal age and retinol concentration. A unit increment in maternal age was associated with 0.01 $\mu\text{mol/l}$ decline in serum retinol concentration. However, the third US NHANES suggested that serum retinol in females' increases during the first 2 decades of life and remains constant in the remaining reproductive ages (277). The difference might be due to the reason that in the developing world repeated nutritionally depleting lifetime events like extended breastfeeding and drought occur to exhaust maternal store across the lifespan. According to EDHS 2005, the prevalence of adjusted night-blindness steadily increased from 4.3% in teenagers to 7.3% in women aged 30-34 years (4). A study in India also demonstrated a twofold increased risk of maternal night-blindness among women aged 35-49 years compared to teenagers (144). In Nepal the prevalence of night-blindness was lower for those in their twenties and rose for women in their thirties (143).

Several studies witnessed the positive influence of household wealth on maternal VA status. According to EDHS 2005, prevalence of night-blindness was elevated by more than twofold in women from poorest households than in the richest households (4). A study in Gondar identified that women with low retinol were likely to live in earth-floored and mud-walled houses, and less likely to own a house (17). Among Nepalese women, night-blindness was more common among lower castes and women who live in poorer quality homes and own no land (28). However in the current study household wealth status was not associated to VAD status. Alike ZD, the unexpected finding might be attributable to the way household index was measured.

The burden of VAD was found to be higher among educationally underprivileged women. Studies conducted in Ethiopia and elsewhere reported the same. According to EDHS 2005, among women who gave birth in the preceding 5 years of the survey, the prevalence of adjusted night-blindness in illiterates was 1.5 and 2.4 times higher compared to women who had primary and secondary or higher level of education, respectively (4). Studies conducted in India (144), and Nepal (145) reported a substantial 50% reduction in the burden of night-blindness among literate women. Better maternal education might have contributed to the reduction of prenatal VAD through enhancing good nutritional awareness and practice prior to and during pregnancy.

Repeated reproductive cycles were associated with increased risk of VAD. This is consistent to the knowledge that it depletes maternal stores (41). Previous studies in developing world concluded likewise. According to EDHS 2005, the prevalences of night-blindness among parity groups of 1, 1-5 and greater than 5 were 4.8, 6.1 and 6.8%, respectively (4). In a study in Southern India multiparas had two times increased risk of maternal night-blindness compared to nulliparas (144). Another study in India documented elevated risk of VAD in women with advanced parity and shorter birth intervals (149). Studies in Cambodia (152) and Nepal (143) found 1.5 and 1.6 times higher risk of night-blindness in women with 3 or more previous births.

VAD and ZD showed a statically significant association. A quarter of the subjects had both of the deficiencies and two-third of VA deficient subjects had ZD. Joint nutritional and non-nutritional causes might not fully explain the association as they had been statistically controlled. The relationship might be secondary to the physiological significance of zinc for the absorption and transportation of VA (164). Empirical studies have also indicated the potential role of ZD as a secondary cause of VAD. In Indonesia higher plasma retinol was observed in pregnant women who received zinc supplementation (173) and in Nepal zinc was found to enhance the effect of VA in restoring night vision among night-blind pregnant women (177).

Distance from the nearby health facility, frequency of ANC and receiving of nutrition education during pregnancy were not related to prenatal zinc and VA statuses. This might be due to the reason that nutritional care is not well integrated into prenatal service. For instance, in the current study among women who had at least one ANC visit, only 76% received nutrition education and 39% were weighed. In addition, the provision of nutrition education might not be effective in the absence of concurrent livelihood and food security promotion strategies.

So far very few studies in Ethiopia attempted to assess the prevalence of LBW. A community-based study in Southwestern Ethiopia reported 10% prevalence (184). A 20 years retrospective study in Tikur Anbessa hospital and a prospective study in four major hospitals of Addis Ababa reported 8.4% and 9.1% prevalences, respectively (185, 186). Two studies in Jimma hospital (187, 188) and studies in Gondar (189) and Metu (190) hospitals reported 11.0%, 22.5%, 15.4% and 8.6% prevalences. In the current study the prevalence of LBW was 16.5%. Compared to most of the aforementioned studies, it witnessed higher prevalence possibly due to the fact that it was exclusively conducted in a rural district. Further, due to the community based nature of the study; it was only possible to weigh newborns within 72 hours of birth. As birthweight declines by 5-7% in the first three days of life (278), the study might have underestimated birthweight with equivalent proportion.

In the linear regression model comprising newborn sex, parity, maternal age, CRP, maternal height and MUAC as predictors, only 20% of the variability in birthweight was explained. The low adjusted r-squared value might be attributable to the reason that prominent determinants of birthweight like maternal weight gain during pregnancy, pre-pregnancy weight and malaria infection were missed from the model. A systematic review showed that in the developing world the aforementioned factors can roughly explain 40% of the variability in birthweight (34).

In the current study male babies weigh more than females by about 100 gm and the risk of LBW was 1.8 times raised in females. Previous studies in Ethiopia concluded likewise. Studies in Addis Ababa (185) and Metu (190) witnessed a significant net difference of 89 and 258g in favor of males. A study in Nigeria identified sex of the baby as the strongest determinant of birthweight compared to other significant predictors including maternal height, age and parity (279). A systematic review concluded that on average, males weigh 150 g heavier than females (34). The biological mechanism how fetal sex affects birthweight is not clear however it is believed to be due to the effect of androgen (34).

The study identified maternal MUAC as a strong correlate of birthweight. Especially in the linear model removing the variable from the equation reduces the adjusted r-squared value by nearly half to 12%. Likewise studies in Nepal (280) and Bangladesh (281) linked low maternal MUAC with 1.5 and 2.0 times increased risk of LBW. Studies in Brazil (282) and India (206) reported significant correlation coefficients of 0.4 and 0.3 between MUAC and birthweight, respectively. A meta-analysis of more than 20 studies also concluded that low maternal MUAC is a significant predictor of LBW with pooled OR of 1.9 (283).

Maternal height emerged as a strong predictor of birthweight. This is parallel to the knowledge that in the developing world roughly 12% of LBW can be due to maternal short stature (34). Other studies also reported the same. According to a study in Nigeria, 41% of the explained variability in birthweight was attributable to maternal height (279). In India 1.5 times increased risk of giving LBW was documented among women who had short stature (193). In UK a significant 390 g weight difference was detected between babies born to mothers whose height was less than 155 cm and greater than 170 cm (284). Though the exact mechanism how maternal height affects birthweight is not clear it might be a mixed expression of genetic and intergenerational effect of malnutrition (181).

In the current study, unlike a reasonable number of previous studies (185, 285, 286), ANC follow up has not been associated with birthweight. This might have to do with the inferior quality of the service in the locality where only 42% and 76% of the ANC attendants received iron-folate supplementation and nutrition education, respectively. In addition, as ethically required, in the baseline survey women who were not on ANC had been given nutrition education and linked with the health care system so that they start ANC. The measures can create misclassification bias and underestimates the potential association between ANC and birthweight.

The linear regression model identified elevated maternal CRP as an important predictor of birthweight. Previous studies have also linked it with prematurity, IUGR and LBW. According to a study in Netherlands, mean CRP levels measured during the first trimester were significantly higher in women who later delivered a growth-restricted baby when compared to controls (287). A nested case-control study in US reported that elevated CRP level in early pregnancy was associated with 2.6 times increased risk of preterm birth (288). A study in Turkey also linked elevated CRP with LBW among preeclamptic women (289). As elevated CRP is an indicator of general infection/inflammation, the result is in confirmation of the finding of a systematic review that in the developing world approximately 4% of the burden of LBW is attributed to general maternal illnesses (34).

The study did not witness a significant association between prenatal zinc status and infants' birthweight. This is against the findings several observational studies conducted worldwide. Till date about 25 observational studies have reported positive association between birthweight and maternal zinc status indicators (36, 110, 290-295). The study setting include developed countries like US, UK, France, Canada and Japan and developing countries like Nigeria, Egypt, Tanzania, India, China, Jordan, Kuwait and Jamaica. Nearly all of the studies reported positive association merely based on bivariate correlation or t-test analyses. Among the studies only three conducted in US (115), Egypt (296) and Tanzania (110) applied multivariate analyses to control potential confounders. Accordingly the overwhelmingly positive association reported in the literature can be due to the effect of confounding as most of the studies did not adequately control potential extraneous variables like general protein energy malnutrition.

Our meta-analysis also did not witness any significant effect of prenatal zinc supplementation on birthweight. Another meta-analysis of 14 RCTs conducted by Cochrane group concluded the same (35). According to it, the pooled difference in the mean birthweight between zinc supplemented and control groups was 10 g and the difference was not statistically significant. The association failed to be significant after stratification was made based on the serum zinc or dietary zinc intake of the study subjects (35).

Once again prenatal VA status has not been associated with birthweight. Previous observational studies came up with mixed findings and most of them were reliant on bivariate analyses. A study in Israel reported that cord retinol and gestational duration significantly explained more than a quarter of the variability in birthweight (37). Studies in India (24), Guatemala (26) and UK (25) similarly found positive correlation between cord retinol and birthweight. A study in India documented that night-blindness was associated with an increased risk of LBW (158). In USA, among HIV positives, 4.6 and 7.0 times increased risks of LBW were reported in women with low and very low VA levels prior to the third trimester (159). However studies in Bangladesh (38) and USA (39), found no significant association. Systematic review of the available observational studies may help to resolve the existing conflicting findings.

VII. Validity and Generalizability

The internal validity of any study is dependent on the suitability of the study design to address the research questions and the appliance of appropriate method to handle extraneous variables. In general the current study included adequate and representative samples of pregnant women and applied reasonably appropriate study designs (cross-sectional and cohort study designs). Based on the conceptual framework, the study had gathered information on wide range of pertinent variables and potential confounders were statistical controlled.

The validity of a study is also reliant on the data quantity and the precision of measurements. In the study various actions, starting from questionnaire designing to supervision, were instated to assure its quality. The tool was pretested and its reliability and content validity had been properly assessed. Data were gathered using qualified, experienced and appropriately trained personnel. During data collection strict supervision was made by the PI and trained supervisors. Maternal and newborn anthropometric measurements were taken using calibrated and pretested scales. Birthweight measurement was done by properly trained personnel and random post-measurement visits were made so as to verifying that the measurement had been carried out as planned.

The micronutrient status of the study subjects was measured using biochemical indicators. Blood sample collection, serum extraction, transport, storage and laboratory analyses were carried out following standard procedures. The laboratory analyses were conducted at EHNRI using qualified personnel. The quality of the analysis was assured through the use of proper controls and blank samples.

The study was carried out in a subsistent rural population with limited access health and social services. The population was dependent on *enset* as staple diet and characterized by high fertility and liability to food insecurity. Though the study was carried out in a district with its own peculiar attributes, the findings can reasonably be informative about the existing nutritional situation in other settings of the country with similar socio-demographic and dietary characteristics. Many other zones of SNNPR are known to have similar profiles.

VIII. Strengths and Limitations

Several major strengths distinguish the current study from previous efforts. In general the study is comprehensive as it deals with various epidemiological aspects of VAD and ZD including prevalence, correlates and effects on birthweight. The study has included adequate and representative subjects and it was focused on pregnant women who are at the highest risk of the deficiencies. Unlike several previous undertakings, the study applied a community-based design which makes it more dependable and less biased. Information on wide range of potential determinants was collected and confounders were adequately controlled.

The other strength of the study emanated from the measurement of multiple micronutrient status indicators in similar group of subjects. This has permitted for the assessment of interaction and co-occurrence among the micronutrients. As the study was entirely dependent on biomarkers, the measurements were objective and it has exposed the full extent of the deficiencies including the subclinical dimensions. The effect of ZD on birthweight has also been complemented with a meta-analysis of RCTs which enhances the comprehensiveness and credibility of the study.

On the other hand the study also suffers from various limitations emanated from feasibility constraints. Essentially it was delimited to the rural kebeles of the district based on the understanding that merely 5% of the area is urban dweller. The prevalence and correlates of the deficiencies were appraised based on a cross-sectional data. Consequently seasonality bias is likely and in few of the reported associations “chicken or the egg” dilemma can’t be entirely ruled-out. The study might also have overestimated the prevalences of the deficiencies as women in the first trimester were largely underrepresented.

Due to feasibility constraints few important predictors of zinc, VA and birthweight were not measured. This might have compromised the comprehensiveness of the statistical models included in the dissertation. For similar reason, zinc and retinol levels were measured only once during pregnancy. This can potentially over or under estimate their association with birthweight as the exposure status to the deficiencies might not be fixed. In addition birthweight was measured to the nearest 100 g accordingly the study might not be able to detect smaller differences that can be attributed to the exposures. The fact that weight was not measured immediately after birth can also make the study to overestimate the prevalence of LBW.

As the community was reliant on subsistent farming, quantification of the exact household monthly income was not feasible. Accordingly, economic status was measured using wealth index scale which has got its own shortcomings. The study has also faced difficulties in precisely measuring maternal and gestational age as the subjects had little formal education. Considerable proportion of the subjects did not remember the exact day of their LNMP. In such instances gestational age was estimated based on fundal height. Maximum effort has also been made to reasonably estimate maternal age from proxy indicators. In addition the dietary intake pattern of the study subjects was assessed mainly using DDS which is not as dependable as weighted food record method.

In the statistical analyses of correlates of ZD and VAD, OR was used as an estimator of RR. This can potentially overestimate the measure of association as the prevalences of the events under consideration are well above 10%.

IX. Conclusion

Micronutrient deficiencies tend to occur together. Considering zinc, VA and iron deficiencies, 70.3% of the pregnant women had at least one of the deficiencies, 32.9% had two or more concomitant deficiencies and 5.1% had all of the three deficiencies.

With the prevalence of 53.0%, ZD is of public health concern in the locality. Key correlates of ZD were household food insecurity, low DD, dependency on maize as a staple diet and low level of consumption of animal source foods. Illiterates and women devoid of self income had increased risk of ZD. Grand multiparity, old age pregnancy and frequent consumption of coffee were negatively associated to zinc status and ZD tend to occur with anemia. Prenatal iron supplementation, frequency of ANC, nutrition education during pregnancy and access to health service were not associated to ZD.

The prevalence of prenatal VAD was 37.9% and an additional 28.7% of the women had marginal status. Accordingly, VAD has severe degree of public health significance in the locality. Advanced gestational age and elevated CRP were negatively associated with serum retinol concentration. Advanced age, inferior maternal socio-economic status, dependence on poorly diversified and plant based diet, ZD and history of too close and too many births were pertinent correlates of VAD. Frequency of ANC and nutrition education during pregnancy were not associated to VAD.

The prevalence of LBW was 16.5%. Prenatal ZD and VAD occurring neither in the second nor third trimester were not significantly associated to LBW. Further, the deficiencies did not show synergetic interaction in causing LBW. Key risk factors of LBW were maternal illiteracy, maternal thinness and stunting, primiparity, female sex of the baby and elevated CRP. Based on the meta-analysis, prenatal zinc supplementation did not show positive effect on birthweight.

X. Recommendation

Policy and Practical Implications:

- Food-based approaches that focus on dietary diversification like backyard gardening and poultry production should be promoted through full engagement of the agriculture and health sectors.
- The existing efforts to improve women's awareness regarding the importance of optimal nutrition prior and during pregnancy should be strengthened through building the capacity of HEWs. Special emphasis should be given in enhancing the nutrition education and counseling skills of frontline health workers.
- Increasing attention should be given for food-based approach as it is known to be more sustainable to address multiple micronutrient deficiencies at a time. It's also a proven strategy for combating LBW.
- The existing voluntary CHPs and model households in the community should be trained and used as nutrition educators and change agents for the betterment of maternal nutrition.
- Strong intersectoral collaboration should be established between the health and agriculture sectors so as to address the root causes of malnutrition in the community. At grassroots level health and agricultural extension workers shall have to follow suit.
- ZD should be combated through a range of short, medium and long-term strategies. Use of household based phytate reduction techniques can be considered as a short-term option; whereas, implementation of agricultural based strategies like dissemination and use of zinc efficient maize strain and zinc containing fertilizers can be taken as medium-term strategies. Such proven interventions against ZD also need to be reflected on the existing NNS.
- The prevalences of prenatal ZD and VAD were found to be elevated amongst illiterates and women devoid of self income. Accordingly, rural livelihood promotion and socio-economic empowerment of women should be taken as long-term solutions for enhancing the nutritional status of women.
- Enhancing the coverage of family planning service shall have affirmative influence in reducing the burden of ZD and VAD. The NNS and the National Guideline for Control and Prevention of Micronutrient deficiencies should also consider birth control as a key strategy for the reduction of multiple micronutrients deficiencies among women of reproductive age.

- Nutritional services should be well integrated into ANC. This includes strengthening of maternal nutrition related ANC components like nutrition education and counseling, expansion of iron-folate supplementation, screening of pregnant women for SAM and linking with supplementary feeding programs.
- Prenatal iron supplementation does not adversely affect zinc status; accordingly, the practice should be continued.

Future Research Direction:

- In Ethiopia national level zinc prevalence study should be conducted so as to assess the extent of the problem in vulnerable subjects like pregnant women and pre-school age children.
- The etiological role of zinc on vitamin A deficiency should be explored through zinc supplementation trials.
- The effect of coffee intake on zinc status should be investigated further through observational and animal model studies.
- Studies targeted at establishing trimester specific cutoff points for defining VA and zinc deficiencies should be conducted among healthy pregnant women with optimal dietary intake.
- The positive association observed between ZD and anemia should be investigated further through zinc supplementation trials.

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Annexes

Annexes

Annex 1: Original papers

Paper I

RESEARCH ARTICLE

Open Access

Prevalence of prenatal zinc deficiency and its association with socio-demographic, dietary and health care related factors in Rural Sidama, Southern Ethiopia: A cross-sectional study

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Abstract

Background: Several studies witnessed that prenatal zinc deficiency (ZD) predisposes to diverse pregnancy complications. However, scientific evidences on the determinants of prenatal ZD are scanty and inconclusive. The purpose of the present study was to assess the prevalence and determinants of prenatal ZD in Sidama zone, Southern Ethiopia.

Methods: A community based, cross-sectional study was conducted in Sidama zone in January and February 2011. Randomly selected 700 pregnant women were included in the study. Data on potential determinants of ZD were gathered using a structured questionnaire. Serum zinc concentration was measured using Atomic Absorption Spectrometry. Statistical analysis was done using logistic regression and linear regression.

Results: The mean serum zinc concentration was 52.4 (+/-9.9) µg/dl (95% CI: 51.6-53.1 µg/dl). About 53.0% (95% CI: 49.3-56.7%) of the subjects were zinc deficient. The majority of the explained variability of serum zinc was due to dietary factors like household food insecurity level, dietary diversity and consumption of animal source foods. The risk of ZD was 1.65 (95% CI: 1.02-2.67) times higher among women from maize staple diet category compared to *Enset* staple diet category. Compared to pregnant women aged 15-24 years, those aged 25-34 and 35-49 years had 1.57 (95% CI: 1.04-2.34) and 2.18 (95% CI: 1.25-3.63) times higher risk of ZD, respectively. Women devoid of self income had 1.74 (95% CI: 1.11-2.74) time increased risk than their counterparts. Maternal education was positively associated to zinc status. Grand multiparas were 1.74 (95% CI: 1.09-3.23) times more likely to be zinc deficient than nulliparas. Frequency of coffee intake was negatively association to serum zinc level. Positive association was noted between serum zinc and hemoglobin concentrations. Altitude, history of iron supplementation, maternal workload, physical access to health service, antenatal care and nutrition education were not associated to zinc status.

Conclusion: ZD is of public health concern in the area. The problem must be combated through a combination of short, medium and long-term strategies. This includes the use of household based phytate reduction food processing techniques, agricultural based approaches and livelihood promotion strategies.

Keywords: Maternal nutrition, Zinc deficiency, Serum zinc concentration

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Background

Zinc is one of the essential trace elements and vital micronutrients with diverse physiologic and metabolic functions [1]. It participates in all major biochemical pathways and plays multiple roles in the cellular proliferation and differentiation [2]. It affects physical growth, immunity, reproductive function and neuro-behavioral development [2,3].

The effects of zinc on maternal health and pregnancy outcomes have been studied in multiple observational and interventional studies [3]. Studies linked the deficiency with a wide range of complications including pregnancy induced hypertension, premature rupture of membrane, placental abruption, prolonged labour, hemorrhage, infections, intrauterine growth retardation, low birth weight, congenital anomalies, increased neonatal morbidity and poor neurobehavioral development [3,4].

Research conducted over years suggested that zinc deficiency (ZD) is widespread public health problem in the world [5]. World Health Organization estimated that ZD affects 31%, 4-73% in various regions, of the world's population [5]. Currently there is scant reliable information regarding the magnitude of ZD in pregnant women [4]. However, studies conducted in Ethiopia [6], Malawi [7], Nigeria [8], India [9], Iran [10] and Indonesia [11] reported high prevalence figures.

In 2004 International Zinc Nutrition Consultative Group (IZiNCG) estimated that 21.1% of Ethiopian population was at risk of inadequate dietary zinc intake [2]. However empirical evidences are scanty. A study conducted in Sidama zone reported 72% prevalence of ZD among pregnant women [6]. Whereas, a study in Addis Ababa found 0% deficiency and 11.3% marginal deficiency among lactating mothers [12].

This study was conducted in rural Sidama zone where seriously high prevalence of ZD was previously reported by relatively small scale surveys [6,13]. The study aims at assessing the prevalence and determinants of maternal ZD.

Methods

Study design

This is community based, cross-sectional, quantitative study with descriptive and analytic designs.

Setting

The study was conducted in January and February 2011 in 18 kebeles of Sidama zone, Southern Ethiopia. The period was selected as it was neither food insecure nor harvest season. A kebele is the smallest administrative unit in Ethiopia comprising approximately 1000 households. Sidama zone is one of the 15 zones of Southern Nations Nationalities Peoples Region (SNNPR) [14]. The zone has population of 2,966,652 and population density

of 430 people/km² [14]. It is characterized by three agro-ecological zones. The lowlands (20%), the midlands (50%) and the highlands (30%) [15]. About 85% of the population livelihood depends on subsistent farming [16]. Major crops grown in the area are *enset* (*Enset ventricosum*), coffee and maize [15]. The average rural household has 0.3 ha of land [16]. In the SNNPR access to health care is limited [17].

Sample size

Sample size adequate for estimating the prevalence of ZD was computed using single proportion sample size calculation formula with the inputs of 95% confidence level, 5% of margin of error, design effect of 2, non-response rate of 10% and expected prevalence of ZD of 72% [6]. Accordingly, sample size of 682 was computed. However, in order to maximize the sample size for the analytic study component, 750 pregnant women were included in the study.

The adequacy of the sample size for investigating the key determinants of ZD (parity, maternal age, and gestational age) was assessed via double proportion sample size calculation formula using an online application [18]. The calculation was made based on the inputs of 95% confidence level, 80% study power and 1:1 ratio between cases and controls. Expected prevalence figures of the exposure factors in cases and controls were taken from studies conducted elsewhere [10,19]. Ultimately, the available sample size was judged to be adequate to study the aforementioned determinants.

Sampling technique

Initially all the kebeles in the zone were listed and stratified into the three agro-ecological zones: lowlands, midlands and highlands. The total sample size was divided to the three strata proportionally to their population size. From each stratum, 6 kebeles were selected at random and the sample size for each stratum was distributed to the kebeles proportional to their population size. Ultimately 750 subjects were selected using systematic random sampling technique. The sampling frame for pregnant women was developed by having a house to house enumeration. Presumptive symptoms of pregnancy (amenorrhea and/or change in the size of uterus) with subsequent pregnant urine test were used to diagnose pregnancy.

Data collection method

A structured and pretested questionnaire used to assess potential determinants of ZD. The parts of the questionnaire on dietary diversity (DD) and household food insecurity level were adopted from Food and Nutrition Technical Assistance (FANTA) indicator guide for Household Dietary Diversity Score (HDDS) [20] and Household Food Insecurity Access Scale (HFIAS) [21],

respectively. Other parts of the tool were developed by the principal investigators (PIs). The content validity of the questionnaire was assessed against the conceptual framework of the study. Reliability of the tool was checked using test-retest method. Questions with less than 0.7 kappa or Pearson coefficient values were removed or revised.

Three trained and experienced enumerators collected the data. Interviews were made at the nearby health posts. The questionnaire was administered in local language. Height and weight were measured using calibrated Seca[®] scales and the measurements were registered to the nearest 0.1 cm and 0.1 kg, respectively. Altitude of the kebeles measured using Magilan[®] GPS system.

Blood sample collection, serum extraction and zinc level determination

Venous blood was collected using plain SARSTEDT Monovette[®] system and stainless steel needles. The blood was allowed to clot for 20 min and consecutively centrifuged at $3000 \times g$ for 10 min. Visibly hemolyzed samples were discarded. Serum was extracted and transferred immediately into screw-top vials. The samples were kept and transported in icebox. The same day the samples were stored frozen at -20°C . Serum zinc concentration was determined at Ethiopian Health and Nutrition Research Institute using Varian SpectrAA[®] Flame Atomic Absorption Spectrometer. Zinc deficiency was defined as a serum zinc level of less than $56 \mu\text{g/dl}$ during the first trimester, or less than $50 \mu\text{g/dl}$ during the second or third trimester [22]

Hemoglobin level was determined at the field using HemoCue Hb 301[®]. Anemia was defined as a hemoglobin level of less than 11.0 g/dl during the first or third trimester or less than 10.5 g/dl during the second trimester [23].

C-Reactive Protein (CRP) determined qualitatively using HumaTex CRP[®].

Data analysis

Data entry, screening and analysis were carried out by the PIs using SPSS 19.0. Descriptive analysis was done using mean, frequency and percentage. Independent *t*-test and one-way Analysis of Variance (ANOVA) used to compare serum zinc levels across categories of independent variables. The assumptions of ANOVA (normal distribution and homoscedasticity of the dependent variable across the categories of the independent variables) were checked to be fulfilled.

Wealth index quintiles (poorest, poorer, middle, richer, and richest) were computed using Principal Component Analysis (PCA). The index was calculated based on ownership of selected household assets, size of agricultural

land, quantity of livestock and materials used for housing construction. PCA was also applied to reduce variables pertaining to maternal workload.

Logistic and linear regression analyses were used to control potential confounders. Independent variables which significantly associated ($P < 0.05$) to the dependent variable in simple regression models were exported to a multiple regression model for adjustment. In addition conceptually important confounders (like CRP status) were also adjusted. The major assumptions of logistic regression analysis (absence of multicollinearity and interaction among independent variables) and linear regression analysis (normally distributed error terms, linear relation between dependent and independent variables, homoscedasticity and absence of multicollinearity) were checked to be satisfied. The fitness of logistic and linear regression models were assessed using Hosmer-Lemeshow statistic and adjusted R squared value, respectively.

Hemoglobin values were adjusted for altitude according to the formulae recommended by Center for Disease Prevention and Control (CDC) [24].

Ethical considerations

The study was conducted in confirmation of national and international ethical guidelines for biomedical research involving human subjects. Ethical clearance was obtained from the institutional review board of Addis Ababa University. Informed written consent was taken from the study subjects. Needle safety procedures were in line with WHO standard. Nutrition education was given to all subjects. Anemic women were given iron-folate supplementation.

Results

Background information about study subjects

Of 750 pregnant women recruited, 700 were willing to take part in the study. Accordingly, the response rate was 93.3%. The altitude of the kebeles included in the study ranged from 1584 to 2763 m Above Sea Level (ASL). Nearly half, 357 (51.0%), of the study participants were sampled from the midlands (1750 to 2300 m ASL); whereas, the remaining 187 (26.7%) and 156 (22.3%) were from highlands ($> 2300 \text{ m ASL}$) and lowlands ($< 1750 \text{ m ASL}$), respectively.

The mean age (\pm -standard deviation) of the respondents was 28.5 years (± 5.5 years). About two-third, 462 (66.0%) were illiterates and more than four-fifth, 561 (80.1%), were housewives. The vast majority were *Sidama* in ethnicity and Protestant Christians in religion. The median monthly household income was 200 birr. The average household size was 4.3 (± 1.8). The average size of agricultural land per household was 0.38 (± 0.19) hectares. (Table 1)

Table 1 Socio-demographic characteristics of study subjects, rural Sidama, Southern Ethiopia, Feb 2011

Variables	Frequency (n = 700)	Percentage
Age (years)		
15-24	158	22.6
25-34	425	60.7
≥ 35	117	16.7
Educational status		
Illiterate	462	66.0
Informal education	4	0.6
1st-4th grade	123	17.6
Higher than 4th grade	111	15.9
Occupation/Livelihood		
Housewife	561	80.1
Petty-trade	84	12.0
Farmer	44	6.3
Others	11	1.6
Ethnicity		
Sidama	679	97.0
Others	21	3.0
Religion		
Protestant	596	85.1
Catholic	60	8.6
Muslim	30	4.3
Orthodox	14	2.0
Marital status		
Married/Living together	696	99.4
Others	4	0.6

Prevalence of zinc deficiency

The mean serum zinc concentration was 52.4 (+/-9.9) µg/dl (95% CI: 51.6-53.1 µg/dl). The value ranged from 26.5 to 77.9 µg/dl. Serum zinc levels for the first, second and third trimesters were 57.2 (+/-9.4), 53.6 (+/-9.8) and 50.1 (+/-9.9) µg/dl, respectively. ANOVA detected significant global difference across the three trimesters ($F = 20.170, P = 0.000$) and between all possible combinations of pared values. Based on simple linear regression analysis, a unit increment in trimester was associated with 3.46 µg/dl decline in serum zinc level ($t = 5.682, 0.000$).

About 53.0% (95% CI: 49.3-56.7%) of the pregnant women were zinc deficient. The prevalence of ZD during the first, second and third trimesters were 46.8, 48.5 and 58.0%, respectively. Even after applying trimester specific cutoff points, women during the third trimester had 1.57 (95% CI: 1.29-1.91) times higher risk of ZD compared to those in the first trimester.

CRP, fasting status and time of sample collection

According to IZiNCG, serum zinc concentration is artificially affected by the presence of acute inflammation/

infection, time of day of blood sample collection and the fasting status of the subjects [2,22]. In this study the association of the aforementioned three factors with serum zinc level was assessed. Fasting was defined as no intake of food or beverage in the preceding 8 h of the sample collection [22]

About 8.4% of the samples were positive for CRP test. The mean zinc levels for CRP positive and negative samples were 49.8 (+/-9.6) and 52.6 (+/-9.9) µg/dl, respectively. The difference was statistically significant ($t = 2.159, P = 0.034$). The mean zinc levels for samples collected in the morning and afternoon were 53.2 (+/-9.9) and 50.1 (+/-10.0) µg/dl, respectively. The difference was also significant ($t = 3.755, P = 0.000$). Nevertheless, the mean zinc concentrations for fasting and non-fasting subjects were 52.3 (+/-9.9) and 52.9 (+/-9.9) µg/dl respectively and the difference was not significant ($t = 1.761, P = 0.079$).

Socio-demographic factors and zinc deficiency

Maternal age was negatively associated to zinc status. The mean serum zinc levels for women aged 15-24, 25-34 and 35-49 years were 53.6 (+/-10.2), 52.2 (+/-9.9) and 51.4 (+/-9.5) µg/dl, respectively. The global difference across the groups was significant ($F = 3.819, P = 0.022$). Compared to pregnant women aged 15-24 years, those aged 25-34 and 35-49 years had 1.57 (95% CI: 1.04-2.34) and 2.18 (95% CI: 1.25-3.63) times higher risk of ZD.

Maternal education and zinc status were positively associated. The mean serum zinc values for illiterates, those who completed 1st-4th grade and beyond 4th grade were 51.6 (+/-9.9), 52.6 (+/-9.5) and 54.9 (+/-9.5) µg/dl, respectively. The global difference was significant ($F = 4.728, P = 0.009$). Compared to women educated beyond 4th grade, illiterates and those who completed 1st-4th grade were at higher risk of ZD with AOR of 1.71 (95% CI: 1.09-2.60) and 1.69 (95% CI: 1.01-2.85).

Household wealth index was not associated to zinc status. Nevertheless, women who were not involved in Income Generating Activities (IGA) had 1.74 (95% CI: 1.11-2.74) times higher risk of ZD than their counterparts.

The associations between ZD and geographical factors like altitude, agro-ecology and kebele were explored. There was no significant difference in serum zinc level across the 18 kebeles ($F = 0.442, P = 0.954$). Similarly, altitude was not correlated to serum zinc level ($r = 0.041, P = 0.277$). The mean serum zinc level and risk of ZD were not significantly different across the three agro-ecological zones (Table 2).

Zinc deficiency and parity

The mean parity was 2.4 (+/-1.7). The mean zinc level for nulliparas was 54.4 (+/-9.8) µg/dl. The corresponding value for parity categories of 1-2, 3-4 and 5 or more were

Table 2 Zinc deficiency and various socio-demographic variables among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011

Variables	Serum zinc µg/dl (mean+/-sd)	ZD+	ZD-	Crude OR (95% CI)	Adjusted OR (95% CI) **
Age (years) (n = 700)					
15-24	53.6 (10.2)	66	92	1 ^r	1 ^r
25-34	52.2 (9.9)	232	193	1.68 (1.14-2.47)*	1.57 (1.04-2.34)*
≥ 35	51.4 (9.5)	73	44	2.31 (1.38-3.89)*	2.18 (1.25-3.63)*
Educational status (n = 700)					
Illiterate	51.6 (9.9)	255	207	1.74 (1.14-2.65)*	1.71 (1.09-2.60)*
1st-4th grade	52.6 (9.5)	70	57	1.74 (1.05-2.90)*	1.69 (1.01-2.85)*
Higher than 4th grade	54.9 (9.5)	46	65	1 ^r	1 ^r
Involvement in IGA (n = 700)					
No	52.1 (9.9)	333	272	1.84 (1.18-2.86)*	1.74 (1.11-2.74)*
Yes	53.8 (10.2)	38	57	1 ^r	1 ^r
Household wealth index (n = 700)					
Poorest	52.6 (9.6)	73	66	1.00 (0.62-1.60)	
Poorer	51.4 (10.3)	74	54	1.24 (0.77-2.01)	
Middle	53.1 (10.2)	78	74	0.93 (0.59-1.47)	
Richer	51.9 (9.4)	72	68	0.96 (0.60-1.53)	
Richest	52.7 (10.1)	74	67	1 ^r	
Agro-ecology (n = 700)					
Highland	52.9 (10.3)	105	82	0.99 (0.64-1.51)	
Midland	52.6 (9.6)	179	178	1.25 (0.86-1.83)	
Lowland	52.9 (10.1)	87	69	1 ^r	

1^r set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables and CRP status

53.4 (+/-9.3), 51.8 (+/-10.1) and 47.1 (+/-9.8) µg/dl, respectively. Based on ANOVA, the global difference across the groups was significant ($F = 10.481$, $P = 0.000$). Post-hoc test identified that those women with 0, 1-2 and 3-4 parity had significantly higher mean serum zinc values compared to grand multiparas. In the logistic model, grand multiparas were found to have 1.7 times higher risk (95% CI: 1.09-3.23) of ZD compared to nulliparas.

Among 553 who gave at least a birth in the previous 5 years of the survey, the association between birth interval and zinc status was assessed. In 55 (7.9%) of the cases the birth interval was less than the recommended 24 months. The mean serum zinc levels for women with short (< 24 months) and optimal (≥ 24 months) birth intervals were 49.3 (+/-7.8) and 52.2 (+/-10.1) µg/dl, respectively. The difference was significant ($t = 2.547$, $p = 0.013$). In logistic regression model, the risk of ZD for women with short birth interval was 1.97 (95% CI: 1.07-4.24) times higher than their counterparts. (Table 3)

Zinc deficiency and dietary factors

The majority, 451 (64.4%), of the respondents considered *Enset* (*Enset ventricosum*) as their staple diet; whereas, cereals (mainly maize) were considered by the

remaining 249 (35.6%) of the respondents. The serum zinc levels in *enset* and maize staple diet categories were 53.3 (+/-9.0) and 50.8 (+/-10.5) µg/dl, respectively. The difference was significant ($t = 3.035$, $P = 0.003$). After adjustments were made for other significant variables, the risk of ZD was 2.25 (95% CI: 1.61-3.16) times higher in maize based staple diet category.

The dietary diversity (DD) level was assessed using 24 h recall method. Respondents were asked whether they had taken any food from predefined 12 food categories in the previous day of the survey. Accordingly, the level of DD was computed out of the score of 12. The mean DDS was 4.3 (+/-1.7). More than one-third, 243 (34.7%), had low DDS. Compared to women with high DDS (DDS ≥ 6 [25]), the risk of ZD was 1.87 (95% CI: 1.02-2.91) and 2.57 (95% CI: 1.57-4.18) times higher among those with optimal (DDS = 4 or 5 [25]) and low DDS (DDS ≤ 3 [25]), respectively.

The mean serum zinc levels for women who consumed and who did not consume animal source in the previous day of the survey were 55.9 (+/-9.9) and 51.2 (+/-9.1) µg/dl, respectively. The difference was significant ($t = 6.088$, $P = 0.000$). The risk of ZD was 2.51 (95% CI: 1.70-3.72) higher among those who did not consume animal source foods in the reference period.

Table 3 Zinc deficiency and reproductive health factors among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011

Variables	Serum zinc µg/dl (mean+/-sd)	ZD+	ZD-	Crude OR (95% CI)	Adjusted OR (95% CI)**
Parity (n = 700)					
0	54.4 (9.8)	52	64	1 ^f	1 ^f
1-2	53.4 (9.3)	138	138	1.23 (0.80-1.90)	0.72 (0.46-1.15)
3-4	51.8 (10.1)	126	105	1.48 (0.94-2.31)	0.77 (0.48-1.24)
≥ 5	47.1 (9.8)	55	22	3.08 (1.66-5.69)*	1.70 (1.09-3.23)*
Birth interval (n = 553)					
6-23 months	49.3 (7.8)	38	17	2.05 (1.13-3.72)*	1.97 (1.07-4.24)*
≥ 24 months	52.2 (10.1)	260	238	1 ^f	1 ^f

1^f set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables and CRP status

The level of household food insecurity was assessed using HFIAS. The scale appraises the occurrence of nine food insecurity related events in the household in the preceding four weeks of the survey. The reporting of an event earned a score of one while the opposite earned zero. Accordingly, the scale was computed out of nine. The higher the score the more serious the household food insecurity problem. Those who scored above the mean value of HFIAS had 5.07 (95% CL: 3.67-6.99) times higher risk of ZD compared to women with below average score.

Coffee drinking is a common cultural practice in Sidama. In order to assess the association between coffee intake and zinc status, respondents were asked to estimate the volume of coffee they usually take (per 70 ml coffee cup) in a typical day. In average every woman takes 3.3 (+/-1.4) cups of coffee per day. Women who reported more than average coffee intake had 1.41 (95% CI: 1.06-1.84) times higher risk of ZD compared to their counterparts. (Table 4).

Zinc deficiency and maternal workload

In order to appraise the relationship between maternal workload and zinc status, the most common and laborious activities in the community were identified and the mean number of the activities in the preceding week of the survey were quantified. Using PCA, an index of five relative ordinal categories (lowest, lower, middle, higher, and highest) was generated. Based on ANOVA, the mean serum zinc level across the categories was not significantly different ($F = 0.509$, $P = 0.729$). Similarly, in the logistic regression model the risk of ZD was not different across the maternal workload categories.

Zinc deficiency, iron supplementation and anemia

Of all respondents, 140 (20.0%) took iron-folate supplement (daily dose of 150 mg ferrous sulphate and 500 µg folate) at least once in the preceding four weeks of the survey. However, only 105 (15.0%) reported full compliance for the supplement in the reference period.

Table 4 Zinc deficiency and dietary factors among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011

Variables	Serum zinc µg/dl (mean+/-sd)	ZD+	ZD-	Crude OR (95% CI)	Adjusted OR (95% CI) **
Staple diet (n = 700)					
Enset based	53.3 (9.0)	217	234	1 ^f	1 ^f
Cereal based	50.8 (10.5)	154	95	1.74 (1.28-2.40)*	2.25 (1.61-3.16)*
Diet Diversity (n = 700)					
Low	50.3 (9.6)	162	81	4.78 (3.04-7.51)*	2.57 (1.57-4.18)*
Optimal	52.0 (9.9)	168	150	2.68 (1.75-4.10)*	1.87 (1.02-2.91)*
High	56.9 (9.0)	41	98	1 ^f	1 ^f
Consumed animal source foods (n = 700)					
No	51.2 (9.1)	312	207	3.12 (2.18-4.45)*	2.51 (1.70-3.72)*
Yes	55.9 (9.9)	59	122	1 ^f	1 ^f
HFIAS (n = 700)					
Below the mean value	56.4 (8.7)	116	241	1 ^f	1 ^f
Above the mean value	51.2 (9.4)	255	88	6.02 (4.33-7.36)*	5.07 (3.67-6.99)*
Frequency of coffee intake per day (n = 700)					
≤ 3 coffee cups	53.1 (9.8)	189	141	1 ^f	1 ^f
> 3 coffee cups	51.6 (10.0)	182	188	1.38 (1.02-1.89)*	1.41 (1.06-1.84)*

1^f set as reference, * Significant association at 95% confidence level, ** adjusted for all significant variables and CRP status

In order to assess the association between iron supplementation and zinc status, the serum zinc level among those who did not take any supplement in the preceding four weeks was compared to those who took all the recommended 28 tablets. The mean zinc levels for the two groups were 52.9 (+/-9.8) and 50.9 (+/-10.0) µg/dl, respectively. The difference was not significant ($t = 1.966, P = 0.051$). Similarly, using logistic regression the risk of ZD was not significantly higher in the iron-folate supplemented group (OR = 0.72 (95% CI: 0.47-1.11)).

The association between the duration of prenatal iron supplementation and zinc status was also assessed. The serum zinc levels for those who took the supplement for less than 4 weeks, 4-8 weeks and more than 8 weeks were 50.4 (+/-9.7), 52.9 (+/-10.2) and 49.6 (+/-10.2) µg/dl, respectively. The difference was not statistically significant ($F = 0.907, P = 0.406$).

About two-third, 221 (31.6%), of the pregnant women were anemic. Anemia and ZD tend to occur together. About 149 (21.3%) of the subjects had both deficiencies. Among anemic women, more than two third, 149 (67.4%), were zinc deficient. Similarly, among zinc deficient women about 149 (40.3%) were anemic. After controlling other significant variables and CRP status, anemia and ZD were associated with OR of 2.37 (95% CI: 1.96-3.33).

Zinc status and health care services

The serum zinc level and the risk of ZD were compared across different variables related to health care service. In logistic model, distance from nearby health facility, number of ANC follow up and receiving nutrition education during the pregnancy were not associated to ZD (Table 5).

Linear modeling of maternal serum zinc level

Linear regression model containing all significant independent variables was developed in order to assess the

total variability of the maternal zinc status explained by the covariates. The independent variables with their respective regression coefficients are given in Table 6.

The covariates explained 53.3% of the total variability of serum zinc level. Out of the explained variability the major share was attributable to nutritional factors (HFIAS, DDS, consumption of animal source foods, type of staple diet and frequency of coffee intake). The removal of the nutritional factors from the model would reduce the adjusted R squared value to 15.6%.

Unit increment in maternal age, gestational trimester, and parity were associated with 0.2, 1.7 and 0.8 µg/dl decline in serum zinc level, respectively. Net adjusted difference of 3.6 µg/dl was witnessed between *enset* and maize staple diet categories. The serum zinc level among those who consumed animal source food in the previous day of the survey was significantly higher by 1.0 µg/dl than their counterparts. DDS and HFIAS were associated to serum zinc level with unstandardized beta coefficients of 2.5 and -0.9, respectively. As the daily frequency of coffee intake increases by one cup, serum zinc declines by 0.5 µg/dl. Net adjusted difference of 1.3 µg/dl was witnessed between samples collected in the morning and afternoon. Serum zinc and blood hemoglobin level were positively associated with regression coefficient of 0.8.

Discussion

The reported prevalence of ZD is prone to seasonality bias as the study is cross-sectional. The study was carried out in January and February which are locally considered as transitional months to household food insufficiency. If it had been conducted in food insecurity prone months (April to July), a higher prevalence would have been expected.

According to IZiNCG, the risk of ZD is considered to be of public health concern when the prevalence of low serum zinc concentrations is greater than 20% [26].

Table 5 The association between zinc deficiency and health care related factors among pregnant women in rural Sidama, Feb 2011

Variable	Serum zinc µg/dl (mean+/-sd)	ZD+	ZD-	Crude OR (95% CI)
One-way walking distance from nearby health facility (n = 700)				
0-30 min	52.5 (9.9)	311	287	0.76 (0.50-1.16)
Longer than 30 min	51.6 (10.2)	60	42	1 ^r
Frequency of ANC follow up (n = 700)				
0	53.4 (9.9)	184	180	1 ^r
1-2	51.3 (9.8)	171	136	1.23 (0.48-2.20)
≥ 3	50.6 (9.5)	16	13	1.20 (0.56-2.58)
Received nutrition education during the pregnancy (n = 700)				
Yes	51.4 (10.2)	99	76	1.21 (0.86-1.71)
No	52.7 (9.8)	272	253	1 ^r

Table 6 The outputs of the linear regression model

Variables	Unstandardized Coefficients	Standardized Coefficients	t	P value
Constant	42.816	-	12.417	0.000
Maternal age (in years)	-0.155	0.087	-2.410	0.016
Trimester (first, second, third)	-1.725	-0.106	-4.232	0.000
Parity	-0.753	-0.132	-3.581	0.000
Staple diet (0 = Enset, 1 = Maize)	-3.601	0.517	-6.971	0.000
Consumption of animal source food (0 = No, 1 = Yes)	0.983	0.475	2.069	0.039
DDS (0-12)	2.539	0.152	16.757	0.000
Frequency of coffee intake/day	-0.528	0.166	-3.192	0.001
Blood hemoglobin level	0.772	0.197	3.917	0.000
HFIAS (0-9)	-0.928	0.123	-7.523	0.000
Time of data collection (0 = Morning, 1 = Afternoon)	-1.282	0.537	-2.386	0.017

Hence, the study signals the public health significance of ZD in the area [1].

Till date two pocket studies in Ethiopia attempted to determine the prevalence prenatal ZD. Both were conducted in Sidama zone [6,13]. According to Abebe et al., in 2004 among 99 pregnant women in third trimester, the prevalence was 72% and the mean plasma zinc was 45.6 µg/dl [6]. According to Hambidge et al., in 2006 among 17 pregnant women in third trimester, the mean plasma zinc was 44.1 µg/dl [13]. The studies reported severe deficiency situation compared to the current study. The variation can be explained by the time gap and seasonal difference in data collection. Further, the studies may have overestimated the problem as they only included pregnant women in the third trimester.

IZiNCG suggested similar cutoffs point (50 µg/dl) for defining ZD during the second and third trimesters [2]. Nonetheless in the current study, parallel to studies conducted elsewhere [10,27], the zinc level in the third trimester was significantly lower than that of the second trimester. This is also consistent with the understanding that hemodilution continues into the third trimester [7,28]. The finding may indicate the need of having different cutoff point for the second and third trimesters.

According to IZiNCG, serum zinc level is artificially affected by CRP status, the time of day of blood sample collection and the fasting status the study subjects [2,22]. However, in current study fasting status was not associated to serum zinc level. Similarly, the serum zinc level differences between CRP positive and negative samples and samples collected in the morning and afternoon were not as huge as observed in NHANES II [22]. The finding signifies that in predominantly zinc deficient community, the effect of the aforementioned three factors may not be as prominent as expected in affluent community.

In the linear regression model the unexplained 46.5% of the variability in serum zinc level might be attributable to range of factors like serum copper level, serum albumin,

intestinal and hemoparasites etc. which were not measured in the study.

Pregnant women from *enset* staple diet category were better-off in their zinc status as compared to women from maize staple diet category. The association could not be explained by dietary diversity and agro-ecological factors as these potential confounders were statistically controlled. The difference can be due to the better bioavailability of zinc in *enset* based diets [29].

Superior household economic standing enhances maternal zinc status [8,11]. However, in this study household wealth index was not associated to zinc status. Nevertheless involvement of women in IGAs is found to have positive influence. This might be due to the reason that in Ethiopia maternal income is usually directly spent to cover household food expenditures.

Two previous studies in Nigeria [8,30] failed to witness any definite trend on the effect of maternal education on prenatal zinc status. However, in the current study maternal education showed positive influence. Higher education status might have contributed to superior zinc status through enhancing good nutritional awareness and practice prior to and during pregnancy.

Maternal age was inversely associated to zinc status. The finding is consistent with the understanding that serum zinc level reaches peak during adolescence and young adulthood, and then declines [31]. Other studies also reported more or less parallel finding [8,10].

The study witnessed the deleterious effect of too many and too close births on zinc status. The finding is parallel to the knowledge that repeated pregnancies deplete maternal store. Previous studies conducted in Malawi [7], Nigeria [8] and USA [32] also supported the finding.

Laboratory and animal model studies indicated that zinc and iron compete for absorption in the intestinal lumen as they have similar physicochemical properties [33-35]. However, many community based studies concluded divergently [10,36-40]. In our study, parallel to studies

conducted in Nigeria [37], Iran [38] and UK [39,40], daily iron-folate supplementation was not associated to maternal zinc status. This might be due to the reason that in the study community the intake of iron and zinc rich foods was low. Hence, competitive absorption which happens at higher concentration of the nutrients might not have taken place.

Hemoglobin and serum zinc levels were positively correlated. The association persisted after adjustments were made for potential nutritional and non-nutritional confounders. Many previous studies concluded likewise [29,41-43]. As the study is cross-sectional, it is not viable to exclude "the chicken or the egg" causality dilemma. However, as zinc is known to participate in multiple metabolic pathways, it might have causal role in anemia.

Chronic overexertion is a predisposing factor to maternal nutritional depletion [44]. However in current study maternal workload was not associated to zinc status. The finding might not be conclusive as level of maternal workload was measured using a relative rather than an absolute scale.

The study found negative association between frequency of coffee intake and zinc status. Coffee is known to contain tannin which can potentially inhibit zinc absorption [45]. However, empirical evidences are lacking. Few available animal model studies concluded divergently [46,47]. Further studies should be conducted in this direction.

Health care service related factors like distance from nearby health facility, frequency of ANC and nutrition education during pregnancy were not related to zinc status. This might be due to the reason that nutritional care is not well integrated into maternity services. In addition, the provision of nutrition education might not be effective in the absence of concurrent livelihood promotion strategies.

Conclusions

Zinc deficiency is of public health concern in the study area. Most of the variability in serum zinc was explained by nutritional factors. Food insecurity, low dietary diversity, dependency on maize staple diet and plant source foods are key predisposing factors to zinc deficiency. Maternal education and involvement in IGAs have affirmative effect. Grand multiparity, old age pregnancy and frequent consumption of coffee were negatively associated to zinc status. Positive association was witnessed between serum zinc and hemoglobin levels. Prenatal iron-folate supplementation does not have negative effect on maternal zinc status. Access to health facilities, ANC follow up and nutrition education were not associated to zinc deficiency.

Recommendation

National level zinc prevalence study should be conducted in Ethiopia. The high prevalence of ZD in the area must

be combated through a range of short, medium and long-term strategies. Use of household based phytate reduction techniques can be considered as a short term option; whereas, implementation of agricultural based strategies like dissemination and use of zinc efficient maize strain and zinc containing fertilizers can be taken as medium term strategies. Rural livelihood promotion and socio-economic empowerment of women will yield the long-term solution. Birth control will also have affirmative input. Nutrition intervention must be strongly inculcated into maternity services. The effect of coffee intake on zinc status should be further investigated. The precise role and contribution of zinc to anemia should be studied.

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Authors' contributions

SG, FE and MU participated in study protocol development, data collection, analysis and write-up. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Paper II

Prevalence and Correlates of prenatal vitamin A deficiency in Rural Sidama, Southern Ethiopia

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Abstract

A cross-sectional study was conducted to assess the prevalence and correlates of prenatal Vitamin A Deficiency (VAD) in rural Sidama, Southern Ethiopia. Randomly selected 700 pregnant women were included. Serum retinol concentration determined using High Performance Liquid Chromatography. Data analyzed using logistic and linear regression. The prevalence of VAD was 37.9%. Advanced gestational age and elevated C-reactive protein were negatively associated with retinol concentration ($P<0.05$). The risk of VAD was significantly higher among illiterates and women devoid of self income. Women aged 35-49 years had 2.23 (95% CI: 1.31-3.81) times higher risk compared to those aged 15-24 years. Women with low dietary diversity score (DDS) were 1.94 (95% CI: 1.17-3.19) times more likely to be deficient compared to high DDS group. With reference to nullparas, multiparas had 2.25 (95% CI: 1.20-4.22) times increased risk. VAD and zinc deficiency were associated with adjusted odds ratio of 1.80 (95% CI: 1.28-2.53). VAD has major public health significance in the area. It should be combated through enhancement of diet diversity, birth control and socioeconomic empowerment of women.

Key words: Maternal nutrition, vitamin A deficiency, serum retinol, nutritional survey.

Introduction

Vitamin A is an essential micronutrient required for the normal functioning of the visual system, immunity, epithelial integrity, cellular differentiation, growth and development and possibly reproduction (1,2). The World Health Organization (WHO) defines VAD as tissue concentrations of vitamin A (VA) low enough to have adverse health consequences even if there is no evidence of clinical deficiency (3).

VAD remains a serious public health problem in the developing world (4). Pre-school children and pregnant women suffer the most widespread and severe effects of VAD (2). According to WHO, VAD is of moderate to severe public health importance in 122 countries (2). Globally 190 million preschool children and 19 million pregnant women have low serum retinol concentration (2). In countries at the risk of VAD, 33.3% of preschool children and 15.3% of pregnant women are deficient (2).

Several studies linked prenatal VAD with various adverse pregnancy and birth outcomes including anemia (5,6), preterm delivery (6-9), intra uterine growth retardation (8,10), low birth weight (5,9,11), malformations (12) infection (13), pre-eclampsia/eclampsia (11,13-15), vertical transmission of HIV (16), poor infant growth (5,11), neonatal and infant mortality (5,17) and maternal mortality (17,18). Nevertheless, the effect of prenatal VA status on birth outcomes is still controversial.

Studies conducted over decades consistently indicated the public health significance of VAD in Ethiopia (19). However, the full extent of the problem in pregnant women is not clearly known as most of the studies were carried in preschool children. Though WHO estimated 13.2% prevalence of VAD in pregnant women in Ethiopia (2), the available three pocket studies (20-22) reported higher prevalence ranging from 17 to 27%.

The purpose of the current study is to assess the prevalence and potential risk factors of prenatal VAD in Sidama zone, Southern Ethiopia, where a previous small-scale survey reported seriously high prevalence.

Materials and Methods

Study design:

This is community based, cross-sectional study with descriptive and analytic designs.

Setting:

The study was conducted in January and February 2011 in 18 kebeles of Sidama zone, Southern Ethiopia. A kebele is the smallest administrative unit in Ethiopia comprising approximately 1000 households. Sidama zone is one of the 15 zones of Southern Nations Nationalities Peoples Regional State (23). The zone has population of 2,966,652 and population density of 430 people/km² (23). Approximately 50%, 30% and 20% of the population dwells in the midlands lowlands and highlands, respectively (24). About 85% of the population livelihood depends on subsistent farming (25). Major crops grown in the area are *enset* (*Enset ventricosum*), coffee and maize (24). The average rural household has 0.3 hectares of land (25).

Sample size:

Single proportion sample size calculation formula was used to determine a sufficient sample size for estimating prevalence of VAD. Accordingly, sample size of 666 was computed based on the inputs of 95% confidence level ($Z_{\alpha/2}=1.96$), 5% of margin of error (d), design effect of 2, expected prevalence (P) of 27% (21) and 10% non-response rate. However, in order to maximize the sample size for the analytic study, 750 pregnant women were included.

The adequacy of the sample size for investigating key correlates of VAD (parity, illiteracy and economic status) was assessed using the STATCALC application of Epi Info version 3.5.1 program. The calculation was made at 95% confidence level, 80% power and 1:1 ratio between cases and controls. The Principal Investigators (PIs) analyzed the Ethiopian Demographic Health Survey (EDHS) 2005 data (on maternal night blindness and associated risk factors) in order to extract important inputs (odds ratio and expected prevalence of exposure in controls) for the sample size calculation (Table 1). Accordingly, the sample size was judged to be optimal for the analytic component of the study.

Table 1: Sample size calculation based on the data of EDHS 2005.

Risk factor	Prevalence of the risk factor in women without night blindness	OR	Expected sample size	
			VAD+	VAD-
Grand multiparty ¹	66.6%	1.80	248	248
Low economic status ²	50.9%	2.69	79	79
Illiteracy ³	89.2%	3.22	230	230

1 Parity of “five or more” compared to parity of “one”

2 “Poorest” wealth index compared to “richest” wealth index

3 “Illiterate” compared to “beyond primary level of education”

Sampling technique:

Initially all the kebeles in the zone were listed and stratified into the three agro-ecological zones: lowlands, midlands and highlands. Then total sample size was divided to each of the three strata proportionally to their population size (20%, 50% and 30% respectively). From each stratum 6 kebeles (total of 18 kebeles) were selected at random. The sample size for each stratum was distributed to the six kebeles proportional to their population size.

In every selected kebele voluntary community health promoters performed house to house enumeration to identify potential pregnant women using presumptive symptoms of pregnancy (amenorrhea and/or change in the size of uterus). Then pregnancy urine test (INSTANT- HCG ®, USA) was done for all women who reported either of the aforementioned symptoms. In every kebele the list of women with positive pregnancy test was developed and used as sampling frame. Ultimately 750 study subjects were selected using systematic random sampling technique.

Data collection method:

A structured and pretested questionnaire used to assess potential determinants of VAD. The part of the questionnaire on dietary diversity (DD) was adopted from Food and Nutrition Technical Assistance (FANTA) indicator guideline (26). Other parts of the tool were developed by the PIs. The content validity and reliability of the questionnaire were assessed. Three trained and experienced enumerators collected the data. Interviews were made at the nearby health posts. The questionnaire was administered in local language.

Blood sample collection, serum extraction and laboratory analysis:

Venous blood was collected using stainless steel needles and plain tubes (SARSTEDT Monovette®, Germany). The blood was allowed to clot for 20 minutes and consecutively centrifuged at 3000×g for 10 minutes. Visibly hemolyzed samples were discarded. Serum was extracted and transferred immediately into screw-top vials. The samples were kept and transported in icebox. The samples were protected from direct sunlight and fluorescent light and were kept frozen at -20 °C until analyzed. Serum retinol and zinc concentrations were determined at Ethiopian Health and Nutrition Research Institute using High Performance Liquid Chromatography (Shimadzu®, Japan) and Flame Atomic Absorption Spectrometer (Varian SpectrAA®, Australia), respectively. C-Reactive Protein (CRP) was determined using qualitative Latex test (HumaTex CRP ®, Germany).

Data analysis:

Data entry, screening and analysis were carried out by the PIs using SPSS 19.0. Descriptive analysis was done using mean and percentage. Independent t-test and one-way Analysis of Variance (ANOVA) were used to compare retinol level across categories of independent variables. The assumptions of ANOVA (normal distribution and homoscedasticity of the dependent variable across the categories of independent variables) were checked to be fulfilled.

Wealth index quintiles were computed using principal component analysis. Logistic and linear regression analyses were used to control potential confounders. Independent variables which were significantly associated ($P < 0.05$) with the dependent variable in simple regression models were exported to multiple regression models for adjustment. The major assumptions of logistic regression analysis (absence of multicollinearity and interaction among independent variables) and linear regression analysis (normally distributed error terms, linear relation between dependent and independent variables, homoscedasticity and absence of multicollinearity) were checked to be satisfied. The fitness of logistic and linear regression models were assessed using Hosmer-Lemeshow statistic and adjusted r-squared values, respectively.

Ethical considerations:

The study was conducted in confirmation of national and international ethical guidelines for biomedical research involving human subjects. Ethical clearance was granted by the institutional review board of Addis Ababa University. Informed written consent was taken from the study subjects. Needle safety procedures were in line with WHO standard. Nutrition education was given to all subjects.

Operational definitions:

1. Vitamin A deficiency: Serum retinol concentration less than 0.7 $\mu\text{mol/l}$ (2).
2. Marginal vitamin A status: Serum retinol concentration of 0.7 to 1.05 $\mu\text{mol/l}$ (2).
3. Zinc deficiency (ZD): Serum zinc level of less than 8.6 $\mu\text{mol/l}$ during the first trimester, or less than 7.6 $\mu\text{mol/l}$ during the second or third trimester (27).
4. Low dietary diversity: DDS less than or equal to three (28).
5. Medium dietary diversity: DDS of four or five (28).
6. High dietary diversity: DDS greater than or equal to six (28).
7. Severe acute malnutrition (SAM): Middle Upper Arm Circumference (MUAC) less than 220 mm (29).
8. Lowlands: Geographical area located below 1750 meters above sea level.
9. Midlands: Geographical area located 1750 to 2300 meters above sea level.
10. Highlands: Geographical area located above 2300 meters above sea level.

Results

Background information about study subjects

Of 750 pregnant women sampled, 700 (93.3%) were willing to take part in the study. Nearly half, 357 (51.0%), of the study participants were from the midlands; whereas, the remaining 187 (26.7%) and 156 (22.3%) were from lowlands and highlands, respectively. The mean age (\pm standard deviation) of the respondents was 28.5 years (± 5.5 years). About 50 (7.2%), 347 (49.6%) and 303 (43.3%) were in the first, second and third gestational trimesters, respectively. About 462 (66.0%) were illiterates and 561 (80.1%) were housewives. The vast majority, 679 (97.0%) and 596 (85.1%), were *Sidama* in ethnicity and Protestants in religion, respectively. The average household size was 4.3 (± 1.8). The average size of agricultural land per household was 0.38 (± 0.19) hectares.

Prevalence of Vitamin A Deficiency

The mean serum retinol level was 0.84 (± 0.41) $\mu\text{mol/l}$ (95% CI: 0.81 – 0.87 $\mu\text{mol/l}$). The levels in the first, second and third trimesters were 0.97 (± 0.40), 0.89 (± 0.42) and 0.76 (± 0.40) $\mu\text{mol/l}$, respectively. The retinol level in third trimester was significantly lower than the corresponding values for the first and second trimesters ($F=11.08$, $P=0.000$). Based on the cutoff point recommended by WHO (2), 37.9% (95% CI: 34.3–41.5%) of the subjects had VAD and an additional 28.7% (95% CI: 25.3 – 32.1%) had marginal VA status (Figure 1). Pregnant women in the third trimester had 2.59 (95% CI: 1.23-5.48) times higher risk of VAD compared to those in the first trimester.

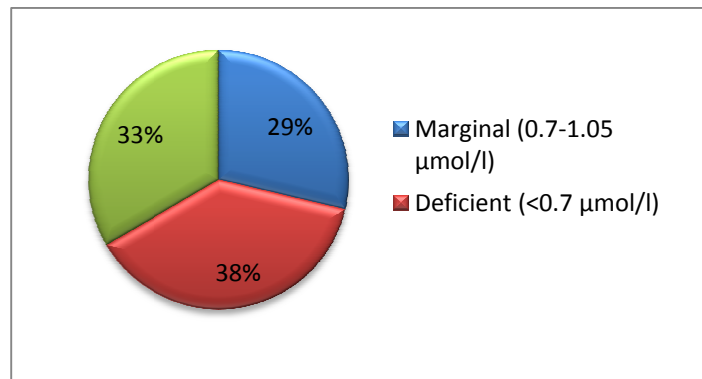


Figure 1: Prevalence of Vitamin A deficiency among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011.

About 59 (8.4%) of the blood samples were positive for CRP test ($\text{CRP} \geq 5 \text{ mg/l}$). The retinol levels for CRP negative and positive samples were $0.85 (\pm 0.42)$ and $0.66 (\pm 0.34) \mu\text{mol/l}$, respectively. The difference was significant ($t=3.828, p=0.000$). Elevated CRP was associated with 22.5% reduction in serum retinol concentration. Positive CRP result was significantly associated with VAD with AOR 2.21 (95% CI: 1.22-4.02).

Risk Factors of Vitamin A Deficiency

Socio-demographic factors

Maternal age was negatively associated with VAD. The retinol concentrations for women aged 15-24, 25-34 and 35-49 years were $0.89 (\pm 0.44)$, $0.85 (\pm 0.40)$, $0.71 (\pm 0.41) \mu\text{mol/l}$, respectively. The global difference was significant ($F=6.89, P=0.001$). The risk of VAD was 2.23 (95% CI: 1.31-3.81) times higher in the oldest age group compared to the youngest.

The study witnessed no significant association between household wealth index and maternal VA status. Nevertheless, women's participation in Income Generating Activities (IGAs) was positively associated. Retinol level among women who were involved in IGA was significantly higher than their counterparts ($t=2.557, P=0.012$). Likewise women who were devoid of self income had 2.18 (95% CI: 1.29-3.69) times higher risk of VAD.

Maternal education showed affirmative influence on VA status. The retinol levels in illiterates, women who completed 1st-4th grade and beyond 4th grade were $0.80 (\pm 0.43)$, $0.87 (\pm 0.38)$, $0.93 (\pm 0.38) \mu\text{mol/l}$, respectively. The global difference across the categories was significant ($F=19.682, P=0.000$). Illiterates had 1.73 (95% CI: 1.02-2.92) times higher risk of VAD compared to women educated beyond 4th grade.

The risk of VAD and the serum retinol level were not significantly different across the three agro-ecological zones represented in the study.

Nutritional Factors

Dietary diversity level was assessed out of a score of 12 based on one day 24 hours recall method. The diet was predominated by root and tubers, cereals and legumes. In the reference period 549 (78.4%), 369 (52.7%) and 366 (52.2%) consumed root and tubers (mainly “*Enset*”), cereals (mainly maize) and legumes (mainly broad bean and kidney bean), respectively. Only 199 (28.4) and 181 (25.6%) reported consumption of provitamin A and preformed vitamin A rich foods, respectively.

Based on ANOVA and logistic regression analyses, level of dietary diversity and consumption of animal source foods in the reference period were positively associated with VA status. Women with low DDS had 1.94 (95% CI: 1.17-3.19) times increased risk of VAD compared to women with high DDS. Those who did not consume animal source foods in the previous day had 1.51 (95% CI: 1.04-2.13) times higher risk than their counterparts.

The retinol level among respondents who considered maize as their staple diet (0.88 ± 0.42 $\mu\text{mol/l}$) was significantly higher than those who reported *enset* (0.81 ± 0.41 $\mu\text{mol/l}$) ($t=2.028$, $P=0.043$). Nonetheless, in the logistic model the association was not significant. Similarly, VA status was not significantly associated with SAM status (Table 2).

VAD and ZD tend to occur together. A quarter, 24.9% (95% CI: 21.7-28.1%), of the subjects had both of the deficiencies and about two-third, 65.6% (95% CI: 59.9-71.4%), of vitamin A deficient subjects were zinc deficient. After controlling joint nutritional and non-nutritional determinants, VAD and ZD were significantly associated with AOR of 1.80 (95% CI: 1.28-2.53).

Reproductive and health care factors

Parity and VA status were negatively associated. Significant difference of retinol concentrations was witnessed across parity categories of 0, 1-2, 3-4 and 5 or more ($F=2.886$, $P=0.035$). Compared to nullparas, multiparas had 2.25 (95% CI: 1.20-4.22) times higher risk of VAD.

Among 553 subjects who gave at least a birth in the preceding five years of the survey, the interval between the previous birth and the current pregnancy was estimated. The retinol levels in women with short (< 24 months) and optimal (\geq 24 months) birth intervals were 0.72 (± 0.45) and 0.84 (± 0.41) $\mu\text{mol/l}$, respectively. The difference was significant ($t=2.032$, $P=0.043$). Women with short birth interval had 2.11 (95% CI: 1.15-3.86) times higher risk of VAD.

The association between duration of breastfeeding and VA status was assessed in 492 women who reported history of breastfeeding in the previous five years of the survey. The retinol concentrations for mothers who breastfed for duration less than 18 months, 18-24 months and greater than 24 months were 0.99 (± 0.42), 0.87 (± 0.42) and 0.79 (± 0.40) $\mu\text{mol/l}$, respectively. The global difference was significant ($F=6.491$, $P=0.002$). Post-hoc Bonferroni test identified that the retinol level among women who breastfed for more than 24 months was significantly lower than those who breastfed for less than 18 months.

Serum retinol level and risk of VAD were compared across various health care service related factors. However, physical distance from the nearby health facility, frequency of antenatal care (ANC) and receiving nutrition education during the pregnancy were not associated with VAD status (Table 2).

Table 2: Association between vitamin A status and various potential risk factors among pregnant women in rural Sidama, Southern Ethiopia, Feb 2011.

Variables	Retinol $\mu\text{mol/l}$ mean ($\pm\text{sd}$)	VAD+	VAD-	Crude OR (95% CI)	Adjusted OR (95% CI) **
Staple diet					
Enset based	0.81 (± 0.41)	180	271	1.28 (0.93-1.77)	-
Cereal based	0.88 (± 0.42)	85	164	1 ^r	-
DDS					
Low	0.75 (± 0.41)	119	124	2.55 (1.63-4.00)*	1.94 (1.17-3.19)*
Medium	0.87 (± 0.41)	108	210	1.37 (0.88-2.12)	1.10 (0.70-1.72)
High	0.91 (± 0.43)	38	101	1 ^r	1 ^r
Consumed animal source foods in the previous day					
No	0.81 (± 0.42)	216	303	1.92 (1.33-2.78)*	1.51 (1.04-2.13)*
Yes	0.91 (± 0.39)	49	132	1 ^r	1 ^r
SAM status					
No	0.87 (± 0.42)	178	330	1 ^r	1 ^r
Yes	0.75 (± 0.39)	87	105	1.54 (1.10-2.15)*	1.26 (0.88-1.81)
ZD status					
Non deficient	0.92 (± 0.41)	91	238	1 ^r	1 ^r
Deficient	0.76 (± 0.40)	174	197	2.31 (1.68-3.17)*	1.80 (1.28-2.53)*
Parity					
0	0.89 (± 0.40)	34	82	1 ^r	1 ^r
1-2	0.85 (± 0.41)	102	174	1.41 (0.86-2.26)	1.14 (0.69-1.87)
3-4	0.84 (± 0.42)	91	140	1.57 (0.97-2.53)	1.35 (0.81-2.26)
≥ 5	0.72 (± 0.40)	38	39	2.35 (1.29-4.28)*	1.92 (1.02-3.64)*
Walking distance from nearby health facility					
0-30 minutes	0.83 (± 0.41)	229	369	1 ^r	-
Longer than 30 minutes	0.89 (± 0.44)	36	66	0.88 (0.57-1.36)	-
Number of ANC					
0	0.87 (± 0.41)	121	243	1 ^r	1 ^r
1-2	0.80 (± 0.43)	134	173	1.56 (1.14-2.13)*	1.42 (0.97-2.02)
≥ 3	0.86 (± 0.41)	10	19	1.06 (0.48-2.34)	0.70 (0.30-1.64)
Received nutrition education during the pregnancy					
Yes	0.80 (± 0.41)	70	105	1 ^r	-
No	0.85 (± 0.42)	195	330	0.89 (0.62-1.26)	-

Linear regression modeling

In the following linear regression model about 26.2% of the variability in serum retinol ($\mu\text{mol/l}$) level was explained by 8 independent variables (Table 3). Unit increment in maternal age (in years), parity and gestational age (in months) were associated with 0.01, 0.04 and 0.03 $\mu\text{mol/l}$ decline in serum retinol level, respectively. On the contrary, a unit increment in DDS (0-12) and middle upper arm circumference (in cm) were associated with 0.04 and 0.02 $\mu\text{mol/l}$ rise in retinol concentration. Serum zinc and retinol concentrations were positively associated with unstandardized beta coefficient of 0.03. An adjusted net difference of 0.13 $\mu\text{mol/l}$ difference was observed between CRP positive and negative samples. Similarly, a significant difference of 0.12 $\mu\text{mol/l}$ was witnessed between those who consumed animal source food in the previous day of the survey and their counterparts.

Table 3: Linear regression output on the predictors of serum retinol concentration ($\mu\text{mol/l}$) during pregnancy in rural Sidama, Southern Ethiopia Feb 2011.

Independent variables	Coefficients		t	P
	Unstandardized	Standardized		
Constant	0.481	-	2.378	0.018
Maternal age (in years)	-0.008	-0.114	-2.474	0.014
Parity (0-9)	-0.036	-0.155	-3.343	0.001
Gestational age (in months)	-0.030	-0.127	-3.730	0.000
Diet diversity level in the previous day (0-12)	0.040	0.162	4.429	0.000
Middle upper arm circumference (in cm)	0.018	0.086	2.515	0.012
Consumed animal source food in the previous day (0=no, 1=yes)	0.117	0.124	3.575	0.000
CRP (0=Negative, 1=Positive)	-0.120	-0.077	-2.339	0.020
Serum zinc concentration (in $\mu\text{mol/l}$)	0.034	0.130	3.545	0.000

Discussion

According to WHO, the public health significance of VAD is considered as severe when the prevalence of low serum retinol ($<0.70 \mu\text{mol/l}$) in pregnant women equals or exceeds 20% (2). Accordingly, VAD is of severe public health importance in the area.

As the study is cross sectional, the reported prevalence figure is prone to seasonality bias. If the study had been conducted in drought prone months like May, June and July, a higher prevalence of VAD would have been anticipated. On the other hand, the study might have overestimated the prevalence as women in the first trimester were underrepresented.

Few pocket studies attempted to determine the prevalence of subclinical VAD among pregnant women in Ethiopia. Gibson et al reported 27% prevalence of VAD among 85 pregnant women in three kebeles of Sidama (21). The prevalence figure is comparable to the current study in the sense that the confidence intervals of the studies overlapped. However, the apparent difference in the point estimators might be due to the fact that the earlier study included convenience sample of women in relatively accessible kebeles.

Two studies conducted among ANC attendants in Gondar hospital, Northwestern Ethiopia, found 17.2% (20) and 18.4% (22) prevalence of subclinical VAD. The current study reported relatively higher prevalence, probably due to the reason that the earlier studies were conducted in an urban setup where prevalence of VAD is expected to be lower. According to EDHS 2005, the prevalence of maternal night blindness in rural areas of Ethiopia was two times higher than the urban areas (30).

Gestational trimester and VA status were negatively associated apparently due to the effect of hemodilution. Parallel findings were reported in Zimbabwe (31), India (32) and Turkey (33). In the current study, serum retinol significantly declined by about 22% from first trimester ($0.97 \mu\text{mol/l}$) to third trimester ($0.76 \mu\text{mol/l}$). A study in Indonesia also reported 20% reduction from first to third trimesters (34). The finding suggests the need of having trimester specific cutoff points for defining prenatal VAD.

Elevated CRP was associated with 22.5% reduction in serum retinol concentration. The finding is consistent to the understanding that inflammation causes reduction in retinol as a consequence of the acute phase response (1,35). A meta-analysis also concluded that elevated CRP protein is associated with 25% decline in retinol level (36). However, it is not viable to exclude a genuine vicious causal association between infection and VAD.

Analysis based on the data of the third US National Health and Nutrition Examination Surveys concluded that serum retinol in females' increases during the first 2 decades and remains constant between 20 and 49 years of age (37). However, in the current study maternal age and VA status were negatively associated. The divergence might be due to the reason that in the developing world repeated nutritionally deleterious lifetime events like extended breastfeeding and drought frequently occur and deplete maternal store across the lifespan. Ethiopian DHS 2005 also found that the prevalence of maternal night blindness tends to increase with age (30). Studies in India (38) and Nepal (39) also concluded likewise.

Unlike many previous findings (38-40), household wealth status was not associated with VAD status. The unexpected finding might be explained based on the reason that household wealth status was measure using a relative rather than an absolute index. Nevertheless, involvement of women in IGAs found to have beneficial effect irrespective of household wealth and maternal education status.

History of too close and too many births were associated with high risk of VAD. This is consistent to the understanding that repeated reproductive cycles deplete maternal store (41). Previous studies in the developing world also reported the same. A study in Southern India reported a twofold increased risk of maternal night blindness among grand multiparas compared to nullparas (38). Studies in Cambodia (40) and Nepal (39) found 1.5 and 1.6 times higher risk of night blindness in women with 3 or more previous births compared to their counterparts, respectively.

VAD and ZD were positively associated. Potential nutritional and non-nutritional confounders can't explain the association as they had been statistically controlled. The association can be secondary to the influence of zinc on the metabolism of vitamin A (42). A study in pregnant rhesus monkeys concluded that in zinc deficient groups zinc affects vitamin A metabolism by altering the formation or release of holo-retinol binding protein (43). Zinc supplementation trial in pregnant Nepalese women also suggested that zinc potentiates the effect of vitamin A in restoring night vision among night-blind pregnant women with low initial serum zinc concentrations (44).

Distance from the nearby health facility, prenatal nutrition education/counseling and frequency of ANC were not associated with vitamin A status. This might be due to the reason that nutritional care is not well integrated into maternity services. In addition, the provision of nutrition education might not be effective in the absence of concurrent livelihood promotion strategies.

Conclusion

The prevalence of VAD was 38%. Accordingly, it has severe public health importance in the area. Advanced gestational age and elevated CRP were negatively associated with serum retinol concentration. Advanced age, inferior maternal socio-economic status, dependence on poorly diversified and plant source based diet, zinc deficiency and history of too close and too many births were pertinent risk factors of VAD. ANC and nutrition education were not potent enough to reduce the burden of VAD. The problem should be combated through improving diet diversity and expansion of family planning service in the area. Socioeconomic empowerment of women will also have positive contribution. Nutritional care should be integrated into maternity services. Trimester specific cutoff points should be developed for defining prenatal VAD.

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Paper III

Independent and joint effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama, Southern Ethiopia: Community based prospective cohort study

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Abstract

Background: The effects of prenatal Zinc Deficiency (ZD) and Vitamin A Deficiency (VAD) on birthweight are controversial and their interaction has not been investigated.

Objective: To assess the independent and interaction effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama, Southern Ethiopia.

Methodology: A community-based prospective cohort study design was employed. Six hundred fifty pregnant women in their second or third trimester were randomly selected and their serum zinc and retinol concentrations were determined. About 575 subjects successfully followed until delivery and birthweight was measured within 72 hours after delivery. The association between the exposures and birthweight examined using log-binomial and liner regression analyses. Potential interaction between ZD and VAD examined using Synergy Index (SI).

Results: The mean birthweight (\pm standard deviation) was 2896g (\pm 423). About 16.5% (95 % CI: 13.5-19.6%) of the babies had Low Birthweight (LBW). Prenatal ZD and VAD were not significantly associated to LBW with Adjusted Relative Risk (ARR) of 1.25 (95 CI: 0.86-1.82) and 1.27 (95% CI: 0.86-1.87), respectively. Stratified analysis on the basis of gestational trimester showed that the occurrence of the deficiencies neither in the second nor the third trimester were associated to LBW. The deficiencies did not show synergetic interaction in causing LBW [SI=1.04 (95% CI: 0.17-6.28)]. Important risk factors of LBW were maternal illiteracy [RR=1.80 (95% CI: 1.11-2.93)], female sex of the newborn [RR=1.79 (95% CI: 1.19-2.67)], primiparity [RR=1.16 (95% CI: 1.02-1.35)], short maternal stature [RR=1.63 (95% CI: 1.06-2.51)] and maternal thinness [RR=1.52 (95% CI: 1.03-2.25)]. In the linear regression model, elevated CRP was also negatively associated to birthweight.

Conclusion: LBW is of public health significance in the locality. The study did not witness any independent or interaction effect of prenatal ZD and VAD on birthweight.

Keywords: Birthweight, zinc deficiency, vitamin A deficiency, maternal nutrition

Background

Low birthweight (LBW) is the single most important predictor neonatal survival and a significant determinant of post-neonatal infant mortality, childhood morbidity and cognitive development [1,2]. The contemporary fetal programming hypothesis linked LBW with markedly increased risk of cardiovascular disease and diabetes in adult life [3]. Globally more than 20 million infants, approximately 15.5% of all births, are born with LBW [4].

In Ethiopia a handful of pocket studies attempted to determine the prevalence of LBW. A community-based study in Southwestern Ethiopia found 10% prevalence [5] and health institution based studies conducted in various urban settings witnessed prevalences ranging from 8.6 to 15.4% [6-11]. The 2000 [12] and 2005 [13] demographic health surveys reported 8% and 14% based on mothers' reporting of birthweight. However the estimates may not be reliable due to recall and reporting errors.

The effects of prenatal zinc and vitamin A deficiencies on birthweight are controversial and their potential interaction has not been studied. Studies endeavored to evaluate the effect of prenatal zinc supplementation on birthweight concluded equivocally [14]. Several observational studies reported positive association between maternal zinc status and birthweight [15]. However, the studies had limited sample size and did not adequately control confounders. Pertaining vitamin A, observational studies in Israel [16], UK [17] and Guatemala [18] in apparently health subjects witnessed positive relation; whereas, studies in Bangladesh [19] and USA [20] found no association.

The purpose of the current study is to investigate the independent and interaction effects of prenatal zinc and vitamin A deficiencies on birthweight in rural Sidama, Southern Ethiopia. The study also assessed the prevalence and general determinants LBW.

The study was conducted based on cohort data of 575 pregnant women in the locality. Earlier, another article [21] based on similar cohort had described the zinc status of the study subjects. Unlike the current article, the previous paper was focused on the magnitude and correlates of prenatal zinc deficiency.

Methods

Study design:

This is a community-based prospective cohort study. Pregnant women were classified based on their prenatal serum zinc and vitamin A statuses and followed until birth. Birthweight was measured within 72 hours of birth.

Study setting:

The study was conducted from January to October 2011 in 18 randomly selected rural kebeles of Sidama zone, Southern Ethiopia. A kebele is the smallest administrative unit in Ethiopia comprising roughly 1000 households. The detail description of the study area has been given elsewhere [21].

Study subjects:

Pregnant women whose zinc and vitamin A statuses were determined at their second or third trimester and who later gave singleton live-births were eligible for the study. Babies visited after 72 hours of birth were excluded. Women whose exposure was determined in the first trimester were also excluded as the fetal weight gain in first trimester is known to be minimal. Flowchart of the cohort from exposure assessment to outcome ascertainment is given in figure 1.

Sample size:

Sample size of 565 pregnant women was considered to be optimal for estimating the prevalence of LBW (birthweight <2.50 kg). The computation was made using single proportion sample size calculation formula with inputs of 95% confidence level, 5% margin of error, 20% expected prevalence of LBW [22], 15% loss to follow-up and design effect of 2. However, in the baseline survey 750 pregnant women were recruited so as to maximize the sample size for the study.

The adequacy of the sample size for investigating the effects of the two exposures on LBW was assessed using double proportion sample size calculation formula. The power calculation was made using STATA/SE 11.0 with the inputs of 95% confidence level, 90% power and 1:1 ratio between exposed and non-exposed subjects. Expected prevalences of LBW in exposed and non-exposed subjects were taken from prior studies [16,23]. As a result, 97 vitamin A deficient and

non-deficient, and 165 zinc deficient and non-deficient subjects were required for the study. Hence the available sample size consisting of 228 vitamin A deficient and 347 non-deficient and 316 zinc deficient and 259 non-deficient subjects was judged to be adequate.

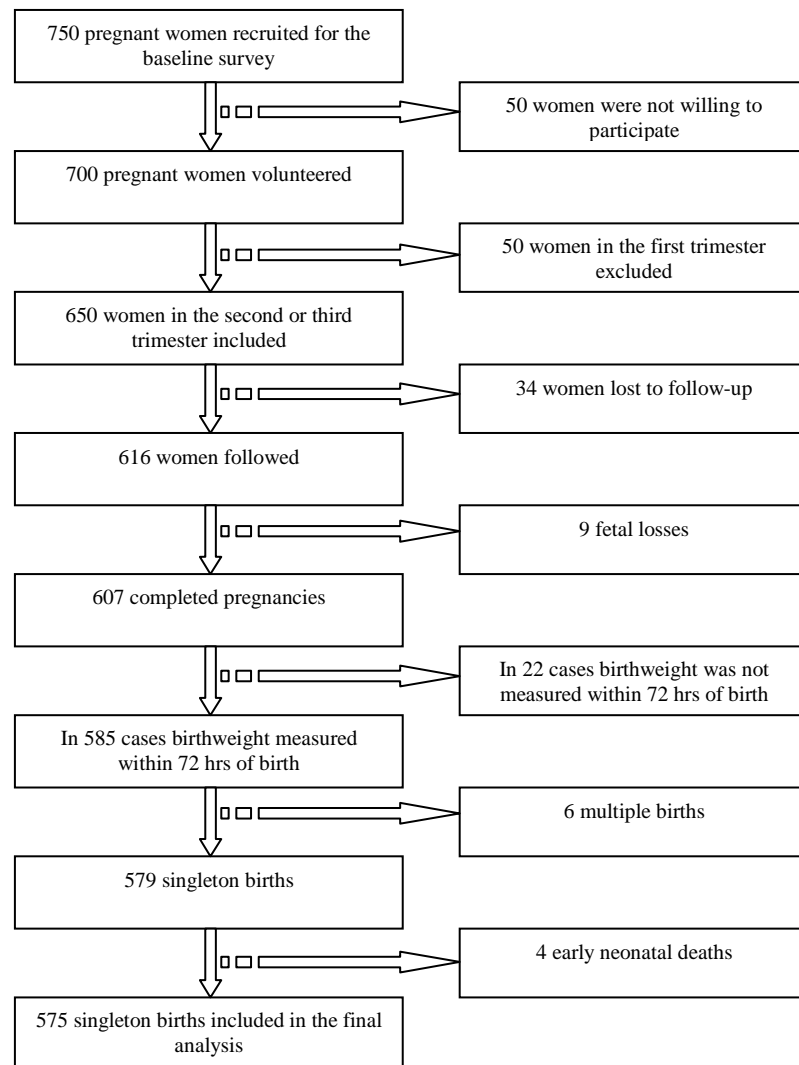


Figure 1: Flowchart of the cohort from exposure assessment to the outcome ascertainment.

Sampling technique:

During the baseline survey 750 pregnant women were selected from the study area using multi-stage sampling technique. The detail description of the sampling method has been given elsewhere [21].

Data collection method:

As described in the previous paper [21], in the baseline survey a structured and pretested questionnaire was used to assess the potential predictors of LBW. Three trained and experienced data collectors gathered the baseline data at the nearby health posts using local language. Maternal height and Mid-upper Arm Circumference (MUAC) were measured to the nearest 0.1 cm using a stadiometer and MUAC tape following standard procedures.

The occurrence of births in the cohort were promptly identified and reported by prearranged local community health promoters and birthweight was measured within 72 hours of birth by trained health extension workers. Weight was measured to the nearest 100 g using calibrated Doebel BRAUN® scales. The data collection activity has been regularly supervised.

Blood sample collection and laboratory analysis:

As described elsewhere [21], venous blood samples were collected, stored, transported and processed following standard procedures. Serum zinc and retinol concentrations were determined at Ethiopian Health and Nutrition Research Institute using Varian SpectrAA® Flame Atomic Absorption Spectrometer, and Shimadzu® High Performance Liquid Chromatography, respectively. C-Reactive Protein level (CRP) was determined qualitatively using latex HumaTex® kit.

Data analysis:

Data entered, screened and principally analyzed using SPSS 19.0. Additional analysis was made via STATA/SE 11.0. Independent t-test and one-way Analysis of Variance (ANOVA) were used to compare birthweight across categories of independent variables. Wealth index quintiles were computed using Principal Component Analysis (PCA).

Linear regression was used to control confounders and to assess the association between the birthweight and various covariates. The fitness of the model was assessed using the r-squared value. Log-binomial regression was also used to model the risk LBW as function of multiple factors. The outputs are given in Crude Relative Risk (CRR) and Adjusted Relative Risk (ARR). The goodness-of-fit assessed using Pearson chi-square and deviance tests.

In the log-binomial model, in accordance with the framework of Kramer [1], the independent variables were entered separately in two blocks of indirect and direct factors. The indirect factors comprised agro-ecological zone and socio-demographic factors where as the direct factors included ZD, VAD, maternal stunting, maternal thinness, CRP status, newborn sex, maternal age and primiparity. In linear regression only the direct factors were modeled as the r-squared value for indirect factors was low. Independent variables that turned out to be significant in bivariate models were considered for multivariate analysis. The assumptions of ANOVA, PCA, linear regression and log-binomial regression were checked.

Potential interaction between ZD and VAD in causing LBW was measured on additive scale. The additive scale is preferred as it is known to be correlated with biological interaction [24,25]. The synergy index was calculated based on Rothman's formula. The 95% Confidence Interval (CI) for synergy index was computed as recommended by Hosmer and Lemeshow [26].

Ethical Considerations:

The study was conducted in confirmation of national and international ethical guidelines for biomedical research involving human subjects. Ethical clearance was secured from the institutional review board of Addis Ababa University. Informed written consent was taken from the study subjects. Nutrition education was given to all subjects and anemic women were given iron-folate supplementation.

Results

Socio-demographic information

Of 650 eligible pregnant women followed, 575 (88.5%) were included in the ultimate analysis. The exclusion was on the bases of loss to follow-up (34), weighed later than 72 hours of birth (22), fetal loss (9), multiple birth (6) and early neonatal death (4). The retained and excluded subjects were not statistically different in terms of wealth index ($P=0.898$), agro-ecological zone ($P=0.284$), literacy ($P=0.802$), CRP status ($P=0.854$), maternal height ($P=0.372$), maternal MUAC ($P=0.763$), prenatal serum zinc ($P=0.056$) and retinol ($P=0.219$) concentrations.

During the baseline survey 304 (52.9%) and 271 (47.1%) of the women were in their second and third gestational trimesters. Their mean age (\pm standard deviation) was 28.5 (± 5.4) years. Nearly half, 299 (52.0%), were from the midlands and the remaining 154 (26.8%) and 122 (21.2%) were from the highlands and lowlands, respectively. About two-third, 381 (66.3%), were illiterates and four-fifth, 462 (80.3%), were housewives. Among babies weighed 289 (50.3%) were males.

Prevalence and general determinants of LBW

The mean birthweight (\pm standard deviation) was 2896g (± 423). About 16.5% (95 % CI: 13.5-19.6%) of the babies were born with LBW.

Maternal literacy showed affirmative influence on birthweight. Weight of babies born to literates (3023g \pm 428) was significantly higher than illiterates (2831g \pm 405) ($t=5.276$, $P=0.000$). The risk of LBW was 1.80 (95% CI: 1.11-2.93) times higher in the earlier group. The mean birthweight increased from 2829g in the poorest to 2951g in the richest wealth quintiles. Yet the difference was not significant ($F=1.899$, $P=0.109$). The prevalence of LBW in the five wealth quintiles were; poorest (23.2%), poorer (19.0%), middle (16.0%), richer (12.6%) and richest (12.3%). However in the log-binomial model the difference was not statistically significant. Likewise women involvement in Income Generating Activities (IGA) was not associated to LBW.

On average, male babies weigh more than females by 103g (95% CI: 34-172g) ($t=2.931$, $P=0.004$) and females were 1.79 (95% CI: 1.19-2.67) times prone to LBW. The weight of babies born to primiparous women (2744g±432) was significantly inferior to those born to parous women (2924g±415) ($t=3.76$, $P=0.000$). The risk was 1.16 (95% CI: 1.02-1.35) times elevated in the earlier group.

The weight of babies born to women who had elevated CRP during pregnancy (2748g±429) was significantly lower than their counterparts (2910g±429) ($t=2.601$, $P= 0.010$). Yet in the multivariate log-binomial model the association was not significant (RR= 1.26 (95% CI: 0.76-2.09)).

Stunting (height <145cm [27]) and thinness during pregnancy (MUAC<220mm [28]) were strong predictors of birthweight. The birthweight of babies born to thin women (2697g±361) was significantly inferior compared to their counterparts (2971g±361) ($t=7.255$, $P= 0.000$). Likewise a 195g (95% CI: 95-294 gm) significant difference was observed between babies born to non-stunted and stunted women. The risk of LBW was 1.63 (95% CI: 1.06-2.51) and 1.52 (95% CI: 1.03-2.25) times elevated in stunted and thin women, respectively.

The risk of LBW was not significantly different across categories of maternal age, agro-ecological zone, antenatal care (ANC), type of staple diet and distance from the nearby health facility (Table 1).

Table 1: General determinants of LBW in rural Sidama, Southern Ethiopia, Jan-Oct 2011.

Variables	Birthweight		Crude RR	Adjusted RR
	Low	Normal		
Women involvement in IGA				
Yes	6	69	1 ^r	1 ^r
No	89	411	2.23 (1.01-4.90)*	2.07 (0.94-4.55)
Literacy				
Literate	19	175	1 ^r	1 ^r
Illiterate	76	305	2.04 (1.27-3.27)*	1.80 (1.11-2.93)*
Wealth index				
Poorest	26	86	1.89 (1.04-3.43)*	1.52 (0.83-2.78)
Poorer	20	85	1.55 (0.83-2.91)	1.27 (0.68-2.41)
Middle	20	105	1.30 (0.69-2.46)	1.13 (0.60-2.13)
Richer	15	104	1.03 (0.52-2.03)	0.93 (0.47-1.83)
Richest	14	100	1 ^r	1 ^r
Distance from the nearby health facility				
0-30 minutes	80	411	1 ^r	-
Longer than 30 minutes	15	69	1.10 (0.66-1.81)	-
Staple diet				
Enset based	59	302	1 ^r	-
Cereals based	36	178	1.03 (0.71-1.50)	-
Sex of the baby				
Male	32	256	1 ^r	1 ^r
Female	63	223	1.98 (1.34-2.94)*	1.79 (1.19-2.67)*
Parity				
Primipara	24	65	1.23 (1.07-1.40)*	1.16 (1.02-1.35)*
Parous	71	415	1 ^r	1 ^r
CRP during pregnancy				
Negative	78	447	1 ^r	1 ^r
Positive	17	33	2.29 (1.48-3.54)*	1.26 (0.76-2.09)
MUAC				
≥ 220 mm	49	368	1 ^r	1 ^r
< 220 mm	46	112	2.08 (1.45-2.99)*	1.52 (1.03-2.25)*
Maternal height				
≥ 145 cm	71	429	1 ^r	1 ^r
< 145 cm	24	51	2.25 (1.52-3.34)*	1.63 (1.06-2.51) *
Maternal age				
15-24	21	106	1.09 (0.58-2.02)	-
25-34	60	296	1.11 (0.65-1.89)	-
35-49	14	78	1 ^r	-
Agro-ecology				
Lowlands	14	108	0.87 (0.58-1.32)	-
Midlands	51	248	0.59 (0.32-1.06)	-
Highlands	30	124	1 ^r	-
ANC during the pregnancy				
Yes	51	231	1 ^r	-
No	44	249	0.83 (0.57-1.20)	-

Maternal zinc and vitamin A statuses and LBW

Standard cutoff points were applied to define prenatal ZD (serum zinc < 7.6 $\mu\text{mol/l}$ [29]) and VAD (serum retinol < 0.7 $\mu\text{mol/l}$ [30]). Using independent t-test analysis, statistically significant 82g (95% CI: 9-150g) and 76g (95% CI: 6-145g) decrease on birthweight was observed in babies born to women who had prenatal vitamin A and zinc deficiencies, respectively. In the bivariate log-binomial model, the deficiencies were also marginally associated with LBW. Nevertheless, in the multivariate model where adjustments were made for potential confounders (sex, parity, CRP status, thinness and stunting), VAD and ZD were not significantly associated to LBW with adjusted RR of 1.25 (95% CI: 0.86-1.82) and 1.27 (95% CI: 0.86-1.87), respectively.

Previous studies witnessed the likelihood of trimester specific effects of micronutrient deficiencies on birthweight [31,32]. In view of that, the log-binomial model was separately executed for women who were at their second and third gestational trimesters during the time of exposure assessment. The occurrence of ZD in the second or third trimester was not associated to LBW with RR of 1.31 (95% CI: 0.73-2.38) and 0.81 (95% CI: 0.46-1.40), respectively. Likewise VAD in second or third trimester was not linked with LBW with RR of 1.26 (95% CI: 0.77-2.07) and 1.35 (95% CI: 0.71-2.57), respectively.

In order to investigate the possibility synergetic interaction of the two deficiencies in causing LBW, the study subjects were categorized into four groups based on vitamin A and zinc deficiency statuses and analyzed accordingly (table 2). VAD and ZD did not show synergetic interaction with synergy index of 1.04 (95% CI: 0.17-6.28).

Table 2: Prenatal zinc and retinol statuses as predictors of LBW in rural Sidama, Southern Ethiopia, Jan-Oct 2011.

Variables	Birthweight		Crude RR	Adjusted RR
	Low	Normal		
Vitamin A status				
Normal	48	299	1 ^r	1 ^r
Deficient	47	181	1.49 (1.03-2.15)*	1.25 (0.86-1.82)
Zinc status				
Normal	34	225	1 ^r	1 ^r
Deficient	61	255	1.47 (1.01-2.16)*	1.27 (0.86-1.87)
Zinc-VA interaction				
Normal zinc and Normal VA	20	155	1 ^r	1 ^r
Zinc deficient and VA normal	28	144	1.42 (0.84-2.43)	1.30 (0.72-2.31)
VA deficient and zinc normal	14	70	1.46 (0.78-2.74)	1.31 (0.66-2.61)
Zinc deficient and VA deficient	33	111	2.01 (1.20-3.34)*	1.76 (1.00-3.11)

Linear modeling of predictors of birthweight

In the bivariate linear regression model altitude of the kebeles, number of ANC visits, diastolic blood pressure during pregnancy were not associated to birthweight ($P < 0.05$). Hence, they were not considered for the multivariate analysis. Sex of the newborn, primiparity, maternal age, serum retinol, serum zinc, maternal height, MUAC and CRP status were exported to the multivariate model as they were significant in the bivariate analysis. In the ultimate model sex, primiparity, height, MUAC and CRP status were significant predictors of birthweight.

The final model explained 20.9% of the variability in birthweight. A cm increment in maternal height and MUAC were associated with 11g and 71g increases in birthweight, respectively. Male babies were 102g heavier than their counterparts. Parous mothers gave 129g heavier babies compared to primiparas. Elevated CRP during pregnancy was linked to 180g decline in birthweight. Serum zinc, retinol and maternal age were not significant predictors of birthweight.

Table 3: Predictors of LBW in rural Sidama, Southern Ethiopia Jan-Oct 2011.

Variables	Unstandardized Coefficients		Standardized Coefficients	t	P
	Beta	SE	Beta		
Constant	-871.3	416.6		-2.092	0.037
Sex (0=Female, 1=Male)	101.7	31.8	-0.120	-3.194	0.001*
Nullyparous (0=Primipara, 1=Parous)	128.7	51.9	0.110	2.481	0.013*
Maternal age (years)	3.7	3.4	0.049	1.091	0.276
Serum zinc ($\mu\text{g}/\text{dl}$)	1.6	1.6	0.037	0.977	0.329
Serum retinol ($\mu\text{mol}/\text{l}$)	-0.1	1.4	-0.003	-0.086	0.932
Body height (cm)	10.8	2.4	0.172	4.458	0.000*
CRP (0=Positive, 1=Negative)	180.2	63.7	0.107	2.830	0.005*
MUAC (cm)	70.5	8.9	0.305	7.941	0.000*

* significant variables in the model

Discussion

LBW is considered as problem great enough to trigger public health action when its incidence exceeds 15% [33]. Consequently, with the incidence of 16.5%, LBW requires focused intervention in the locality.

Due to the community-based nature of the study it was only possible to weigh newborns within 72 hours of birth. As birthweight is known to decline by 5-7% in the first three days of life [34], the study might have underestimated the birthweight with equivalent fraction.

In Ethiopia very few pocket studies attempted to determine the prevalence of LBW. Most were conducted in major referral hospitals. The reported prevalence figures ranged from 8.6 to 15.4% [5-11]. The current study witnessed relatively higher prevalence probably due to the reason that it was conducted in rural area where the burden of the problem is expected to be high.

The linear regression model explained merely 20% of the variability in birthweight. This might have happened as some of the key predictors of low birthweight like weight gain during pregnancy, pre-pregnancy weight and malaria during pregnancy were missed from the model. A study showed that in the developing world the aforementioned factors can roughly explain 40% of the variability of birthweight [1].

Several studies witnessed the positive contribution of superior household wealth status in reducing the burden of LBW [5,35]. However, it was not the case in the current study. The unanticipated finding might have to do with the fact that wealth was quantified using a relative scale as measurement based on actual household income was not feasible. The relative scale might not be powerful when the wealth status of the population is reasonably homogenous.

In the current study male babies weigh more than females by about 100 gm and the risk of LBW was 1.8 times raised in females. Previous studies in Ethiopia and abroad concluded likewise. Studies in Addis Ababa [9] and Metu [11] found net difference of 89 and 258g, respectively. A systematic review concluded that on average, males weigh 150 g heavier than females [36].

Both in the log-binomial and linear regression models maternal MUAC was a strong correlate of birthweight. Especially in the linear model, removing the variable from the equation reduces the r-squared value from 21% to 12%. Even the study might have underestimated the association as pregnant women are commonly enrolled into supplementary feeding programs based on their MUAC. Studies in India [37] and Brazil [38] also reported significant correlation between the two measurements ($r=0.3$ and 0.4 , respectively). A study in Bangladesh linked low maternal MUAC with 1.5 times increased risk of LBW [39].

Maternal height was also a strong predictor of birthweight. This is consistent to the understanding that in the developing world approximately 12% of low birthweight can be explained by maternal short stature [1]. A study in India witnessed that maternal height less than 145 cm was significantly associated to LBW with RR of 1.51 [35]. Likewise a study in UK found 390g birthweight difference between babies born to mothers whose height was less than 155 cm and greater than 170 cm [40]. Though the exact mechanism how maternal height affects birthweight is not clear, it might be the mixed manifestation of genetic and intergenerational effect of malnutrition [36].

Several studies witnessed the beneficial effect of ANC in reducing the burden of LBW [9,41,42]. A study in Addis Ababa found that birthweight in babies born to mothers who attended ANC was significantly increased by 94g [9]. In Mexico women with inadequate number of ANC visits had 63% and 51% greater odds of IUGR and premature delivery [42]. Nevertheless, the current study did not observe parallel findings. This might have to do with the inferior quality of ANC in the locality where only 42% and 76% of the ANC attendants received iron supplementation and nutrition education, respectively.

In the linear regression model elevated CRP during pregnancy was an important predictor of birthweight. The finding is in confirmation of the result of a systematic review that in the developing world approximately 4% of the burden of LBW is attributed to general illnesses during pregnancy and an extra 10% can be linked to malaria [1].

The study did not witness significant association between prenatal zinc status and infants' birthweight. The finding is in confirmation of the conclusion a meta-analysis that maternal zinc supplementation does not enhance birthweight [14]. However several observational studies [15] reported positive association between maternal zinc status and birthweight. This might be due to confounding as most of the studies did not adequately control potential confounders like general protein energy malnutrition.

Observational studies on the relationship between maternal vitamin A and birthweight in apparently health subjects concluded divergently. A study in Israel reported that cord retinol along with gestational age explained more than a quarter of the variability of birthweight [16]. Studies in UK [17] and Guatemala [18] found a significant positive correlation and a study in India linked maternal night-blindness to LBW. However in the current study, parallel to studies conducted in Bangladesh [19] and USA [20], no significant association was witnessed. Systematic review of the available observational studies may help to resolve the existing conflicting findings.

The fact that maternal zinc and retinol concentrations were measured only once during the entire pregnancy can potentially over or under estimate their association with birthweight as the exposure status to the deficiencies might not be fixed. Nevertheless, among women who were at their second gestational trimester during the baseline survey, follow-up survey conducted during their third trimester showed no significant change in their dietary diversity, food frequency and consumption of animal source foods. This might be taken as supporting evidence that their exposure status had not been assorted remarkably.

Conclusion

The prevalence of low birthweight was 16.5%. LBW is of public health significance in the locality. Prenatal vitamin A and zinc deficiencies occurring in the second or third trimesters were not associated with increased risk of LBW. Similarly, the two deficiencies did not show synergetic interaction in causing LBW. Important risk factors of low birthweight were maternal thinness and stunting, primiparity, female sex of newborn and elevated CRP.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

SG, FE and MU participated in study protocol development, data collection, analysis and write-up. All authors read and approved the final manuscript.

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Paper IV

A Systematic Review of Effect of Prenatal Zinc Supplementation on Birthweight: Meta-analysis of 17 Randomized Controlled Trials

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ABSTRACT

The effect of prenatal zinc supplementation on birthweight is controversial as randomized controlled trials (RCTs) report conflicting conclusions. A systematic review which includes meta-analysis was done on 17 RCTs conducted worldwide since 1984 to assess the effect of prenatal zinc supplementation on birthweight. The studies were identified through web-based search. Heterogeneity among studies was assessed using Cochrane Q test statistic. Effect-size was measured based on standardized mean difference. Pooled effect-size was computed using a variant of random effect model. Thirteen of the 17 RCTs found no association, three reported positive association, and one reported negative association. Based on fixed and random effect models, the pooled effect-sizes were 0.0268 [95% confidence interval (CI) 0.0764, -0.0229] and 0.0712 (95% CI 0.1619, -0.0194) respectively. The effect-size estimate remains insignificant after stratification was made based on the dose of supplementation (optimal vs high dose), type of study (community vs institution-based), and type of source country (developed vs developing). The meta-analysis did not witness any association between birthweight and prenatal zinc supplementation.

Key words: Birthweight; Medical research; Meta-analysis; Impact studies; Randomized control trails; Systematic review; Zinc supplementation

INTRODUCTION

Zinc is one of the essential trace elements and a member of the major micronutrients which have attained prominence in human health and nutrition (1). It is required for many biological functions, including DNA synthesis, cell division, gene expression, and stabilization of molecular structures. It is also vital for the activity of more than 300 enzymes participating in the metabolism of macronutrients, micronutrients, and nucleic acids (2,3). Since its importance for human physiology was identified in 1963, many studies witnessed its crucial importance for immune function, linear growth and gain in weight, and neuropsychological and cognitive functions of human beings. Its involvement in such diverse and fundamental activities probably accounts for its essentiality for all forms of life (3).

Poor maternal zinc status is associated with adverse pregnancy outcomes as zinc is assumed to be essential for normal foetal growth and development (4). Animal experiments indicate that severe prenatal zinc deficiency is associated with spontaneous abortion and congenital malformations (5) whereas milder forms are attributed to low birthweight (LBW), intrauterine growth retardation (IUGR), and preterm delivery (6). Difficult and prolonged labour, haemorrhage, uterine dystocia, and placental abruption have also been documented in female rats fed zinc-deficient diets throughout pregnancy (7). The poor pregnancy outcomes in women with acrodermatitis enteropathica are also consistent with effects observed in zinc-deficient pregnant animals (8). However, studies that attempted to evaluate the effect of prenatal zinc status on maternal health and pregnancy outcomes in apparently healthy individuals yielded conflicting conclusions (9).

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As to the effect of prenatal zinc supplementation on anthropometric indicators is concerned, of more than 20 RCTs conducted so far, few reported positive association between zinc supplementation and anthropometric measurements. According to a study in India, birthweight of infants born to women in the placebo group averaged only 2.6 kg

(10). Infants born to zinc-supplemented mothers were 0.3-0.8 kg heavier, depending on the length of time supplemental zinc was provided for. If zinc supplementation was initiated in the first trimester, the effect on birthweight would be greater than if it was initiated in the third trimester (10). A study in the USA among disadvantaged African-American women reported that supplemented zinc increased birthweight by 126 g and increased head-circumference of infant by 0.4 cm (11). Another study in the USA (12) and a study in Chile (13) reported that zinc supplementation enhanced birthweight significantly—approximately by 150 and 69 g respectively. A study in Iran found significantly higher head-circumference of newborns in the supplemented group than in the control group (35.0 cm vs 33.7 cm) but no increments in birthweight (14). However, the remaining RCTs failed to witness any association between anthropometric indicators and prenatal zinc supplementation (15-25).

The objective of this systemic review and meta-analysis was to examine the effect of prenatal zinc supplementation on birthweight. A similar meta-analysis was published in 2009 by the Cochrane Collaboration Group (26). However, this analysis has included four more additional studies and provided the results based on both fixed and random effect models.

MATERIALS AND METHODS

Study design

This is a systematic review which includes a meta-analysis.

Inclusion and exclusion criteria

To assure the quality of analysis, only RCTs were included in the analysis. Articles written in only English language were considered. The exclusion criteria were set based on multiple criteria, including low dose of supplementation (less than 15 mg/day), unavailability of vital information in the articles (mean and standard deviation of birthweight in intervention and control arms, percentage of LBW babies in both arms) and low level of compliance (<70%) for the supplement.

Search strategy and evaluation of studies

Studies were mainly identified through web-based descendent (identifying key literature and look online the other studies which cite them) and ancestor (look into the references of a key article) search techniques. The study did not involve any manual search of articles or contacts with authors. The elec-

tronic search was performed within the Cochrane Library and MEDLINE databases. Further studies were identified using the Google Scholar search engine. Key combination search terms were “zinc supplementation and birthweight” and “zinc supplementation and birth outcomes”. Literature written in only English language were considered, and no limit was made on date of publication of the articles.

Initially, 21 (20 published and 1 PhD dissertation) articles and abstracts based on RCTs were located. The principal investigators reviewed all of them using the predefined inclusion criteria. They also checked quality of the studies in terms of reasonable level of compliance for the supplementation (>70% compliance), follow-up (<30% loss to follow-up), and assuring the comparability of the intervention and control groups based on key variables (maternal age, educational status, maternal anthropometric indicators, gestational age, parity, etc.). Disagreements were solved by repeated evaluations and discussions.

Three of the 21 RCTs, conducted in Germany (27), USA (28) and Denmark (29), were excluded as vital information, such as mean and standard deviation of birthweight in intervention and control arms and proportion of LBW babies in both arms, could not be extracted from the articles and abstracts. A study in South Africa was also excluded since it used very low dose (4.3-12.9 mg/day) of zinc supplementation (30). Hence, the analysis was done with the remaining 17 studies.

From each of the studies included, information on total number of zinc-supplemented and control children, number of LBW babies (<2,500 g), mean birthweight and standard deviation (SD) for both arms, nature of the population, and dose and duration of supplementation were extracted.

Analysis of data

The description of original studies was made using frequency and forest plot. Heterogeneity among studies was statistically assessed using Cochrane Q test statistic. The test statistic indicated random heterogeneity among studies ($p=0.09$). Hence, random effect model was used in the analysis. Among studies, variation was assessed using DerSimonian and Laird's (DL's) estimator. To control the effect of dose of supplementation (optimal or high dose), type of the study (community or institution-based), and type of source country (developed or developing), stratified analysis was made. We analyzed data using Metaeasy add-in for the MS Excel software (version 1.0). As relatively fewer articles were included

in the analysis, Funnel plot was not used for assessing publication bias.

The strength of association between zinc supplementation and birthweight was assessed using effect-size which measures the strength or magnitude of difference between two sets of data (in this case treatment groups). It is the difference between the mean values of the two groups, divided by the standard deviation. The larger the effect-size, the greater is the difference or impact of an intervention. Cohen proposed operational definitions of 0.2, 0.5, and 0.8 as small, medium, and large effect-sizes respectively (31).

RESULTS

Description of original studies

Four studies from the USA, three from the UK, six from Asian countries, three from Latin American countries, and only one from Africa were included in the analysis. In total, these studies involved 6,209 pregnant women in intervention and control arms. The sample-size of the studies ranged from 1,075 in Nepal (32) to 52 in the UK (18). With the exception of six community-based studies, all were health institution-based. All the studies randomized the study subjects into intervention and control groups around the mid-point of the second trimester (16-20 weeks). The dose of the supplement in 12 studies was 15-25 mg/day. However, in the remaining five studies, a higher dose (25-62 mg/day) of supplementation was used. All the studies were published in the last 25 years (1984-2009). The basic information of each specific study is presented in Table 1.

Pooled effect-size

The effect-size estimate of the specific studies showed that the majority (n=13) of the studies found no association between zinc supplementation and birthweight. Three studies reported positive association while one study reported negative association. Based on fixed and random effect model assumptions, the mean (pooled) effect-sizes were 0.0268 (95% CI 0.0764, -0.0229) and 0.0712 (95% CI 0.1619, -0.0194) respectively. In both the cases, the meta-analysis showed no significant association between birthweight and zinc supplementation. The specific and pooled effect-sizes are presented in the figure.

To control the possible effect of confounders, stratification was made based on type of country (developed or developing), dose of supplementation

(optimal or high dose), and type of study (health institution-based or community-based). The classification into developed or developing country was made according to the World Bank cut-off point of gross national income per capita of US\$ 3,945 (33). The mean effect-sizes, based on the fixed and random effect models for the respective categories, are summarized in Table 2.

In all the categories, the Q test statistic was 0.5-0.05 and, as such, the random effect model was taken as the appropriate estimator of pooled effect-size. The stratification indicated that prenatal zinc supplementation was not associated with birthweight, irrespective of dose, type of study, and country.

DISCUSSION

Another meta-analysis of 14 RCTs conducted by Cochrane group also failed to show any association between prenatal zinc supplementation and birthweight (26). According to this study, the pooled difference in the mean birthweight between the zinc supplemented and the control group was -10.59 g (95% CI -36.71, 15.54). Of 10 studies conducted in populations with low serum zinc or low dietary zinc intake, the pooled difference in the mean birthweight between the zinc-supplemented and the control group was -11.42 g (95% CI -38.82, to 15.98) (26). Of three studies conducted in population with normal zinc status or adequate dietary zinc intake, the pooled difference in the mean birthweight between the zinc-supplemented and the control group was -2.32 g (95% CI -88.94, to 84.30). In both the strata, zinc supplementation had no association with birthweight (26).

However, the finding of the two meta-analyses was not consistent to what is documented by many observational studies. In Japan, Higashi A *et al.* have demonstrated that maternal serum zinc level in the second trimester was not related to birthweight of infants (34); however, zinc status in the third trimester was an important determinant. A case-control study in Tanzania reported that mothers with low zinc levels were 2.6 times more at risk of having LBW babies compared to those with normal zinc levels, and newborns with low zinc levels were 2.8 times more at risk of being born with low weight (35). A study in the USA also found that, among both white and black mothers, serum zinc concentration was significantly related to birthweight after various independent determinants of birthweight were controlled (36). A similar association was also documented by studies in Turkey (37), India (38), and China (39).

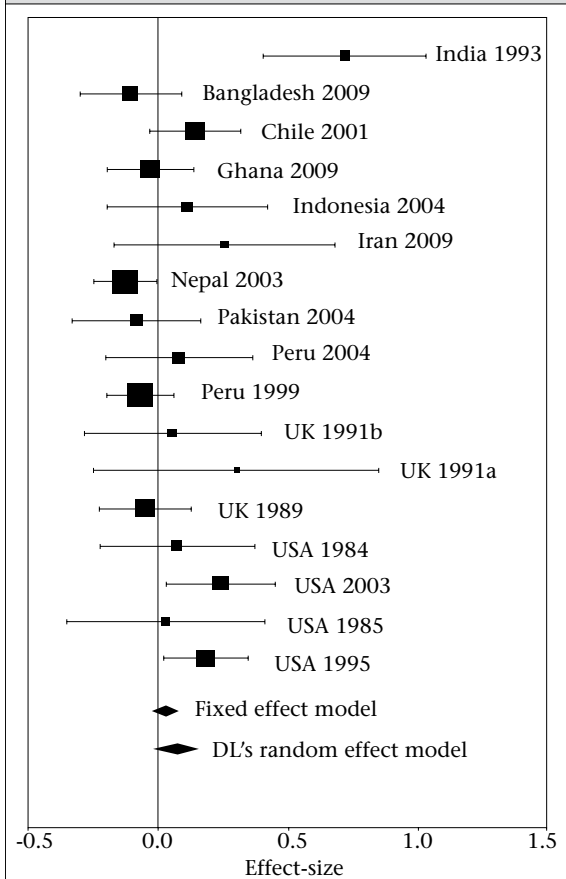
The discrepancy between the conclusion of this analysis and the findings of many observational

Table 1. Description of 17 randomized controlled trials on the association between prenatal zinc supplementation and birthweight, 1984-2009

Author and year of publication	Country	Dose	Type	Intervention Group		Control group		Total sample-size	
				Mean birthweight (g) (SD)	Sample-size	Mean birthweight (g) (SD)	Sample-size		Mean difference (g) (95% CI)
Goldenberg RL <i>et al.</i> , 1995 (11)	USA	25 mg	HIB	3,214 (669)	294	3,088 (728)	286	126 (12.1, 239.9)	580
Hunt IF <i>et al.</i> , 1985 (15)	USA	20 mg	HIB	3,352 (430)	55	3,338 (592)	51	14 (-184.3, 212.3)	106
Tamura T <i>et al.</i> , 2003 (12)	USA	25 mg	HIB	3,267 (592)	173	3,117 (666)	182	150 (19.1, 280.9)	355
Hunt IF <i>et al.</i> , 1984 (16)	USA	20 mg	HIB	3,430 (509)	81	3,368 (442)	64	62 (-93.0, 217.0)	145
Mahomed K <i>et al.</i> , 1989 (17)	UK	20 mg	HIB	3,291 (581)	247	3,319 (531)	244	-28 (-126.4, 70.4)	491
Simmer K <i>et al.</i> , 1991 (18)	UK	23 mg	HIB	2,990 (540)	30	2,820 (600)	22	170 (-146.5, 486.5)	52
Robertson JS <i>et al.</i> , 1991 (19)	UK	62 mg	HIB	3,264 (722)	72	3,220 (871)	62	44 (-229.5, 317.5)	134
Caulfield LE <i>et al.</i> , 1999 (20)	Peru	15 mg	CB	3,267 (461)	488	3,300 (498)	469	-33 (-93.9, 27.9)	957
Meriardi M <i>et al.</i> , 2004 (21)	Peru	25 mg	CB	3,351 (427)	94	3,319 (389)	101	32 (-82.9, 146.9)	195
Hafez A <i>et al.</i> , 2004 (22)	Pakistan	20 mg	HIB	3,023 (456)	121	3,061 (444)	121	-38 (-151.4, 75.4)	242
Christian P <i>et al.</i> , 2003 (32)	Nepal	25 mg	CB	2,598 (428)	553	2,652 (429)	522	-54 (-105.3, -2.7)	1,075
Danesh A <i>et al.</i> , 2009 (14)	Iran	30 mg	HIB	2,961(498)	42	2,819 (609)	42	142 (-95.9, 379.9)	84
Dijkhuizen MA <i>et al.</i> , 2004 (23)	Indonesia	25 mg	CB	3,200 (500)	84	3,150 (400)	80	50 (-88.3, 188.3)	164
Saaka M <i>et al.</i> , 2009 (24)	Ghana	40 mg	HIB	3,105 (490)	272	3,120 (486)	271	-15 (-97.1, 67.1)	543
Duran CC <i>et al.</i> , 2001 (13)	Chile	20 mg	CB	3,319 (460)	249	3,250 (514)	258	69 (-15.8, 153.8)	507
Osendarp JM <i>et al.</i> , 2009 (25)	Bangladesh	30 mg	CB	2,513 (390)	194	2,554 (393)	216	-41 (-116.9, 34.9)	410
Garg HK <i>et al.</i> , 1993 (10)	India	45 mg	CB	3,000 (513)	106	2,600 (541)	62	400 (233.6, 566.4)	168

CB=Community-based; CI=Confidence interval; HIB=Health institution-based; SD=Standard deviation

Fig. Forest plot of 17 randomized controlled trials on the association between prenatal zinc supplementation and birthweight, 1984-2009



studies cannot be explained by non-compliance to zinc supplementation as all RCTs included in this

other reason might be related to the bioavailability of zinc supplement. As absorption of dietary zinc can be inhibited by iron and phytate intake, the same might occur to zinc supplement. Hence, zinc level adequate to promote birthweight may not be achieved after the supplementation of zinc. This hypothesis is also supported by the findings of Hunt *et al.* in the USA (40). According to them, zinc supplementation among pregnant Hispanic women did not alter the mean zinc levels in serum or hair and did not increase the serum zinc level significantly compared to the pre-supplementation level (40).

As meta-analysis heavily depends upon published studies which are more likely to report significant results, non-significant studies which end up in the desk-drawer instead of the public domain would be systematically avoided. However, in this analysis, the problem of publication bias would be less significant as the focus of the analysis is a contemporary issue of scientific debate by which reporting any direction of association would be reasonably interesting to researchers and publishers.

Conclusions

The study did not witness any association between birthweight and zinc supplementation. However, the finding is not conclusive as possible confounding factors were not controlled. This does not mean that zinc status is not a possible predictor of birthweight as zinc supplementation status may not perfectly correlate with serum zinc level. The bioavailability of supplemental zinc should also be investigated.

Table 2. Mean effect-size in different categories of studies: 17 randomized controlled trials on the association between prenatal zinc supplementation and birthweight, 1984-2009

Stratifying variable	Total sample-size of studies	Effect-size with 95% CI (fixed effect model)	Effect-size with 95% CI (random effect model)
Type of country			
Developed	1,863	0.107 (0.197, -0.017)	0.107 (0.199, -0.016)
Developing	4,345	-0.008 (0.051, -0.068)	0.057 (0.183, -0.070)
Type of study			
Community-based	3,476	0.101 (0.174, 0.026)	0.129 (0.267, -0.010)
Health institution-based	2,732	-0.031 (0.041, -0.103)	0.075 (0.268, -0.118)
Dose of supplementation			
15-25 mg/day	4,869	0.016 (0.072, -0.040)	0.040 (0.123, -0.043)
26-62 mg/day	1,339	0.067 (0.175, -0.041)	0.169 (0.457, -0.119)

CI=Confidence interval

analysis reported a satisfactory level of compliance. One possible reason might be the risk of potential confounding bias in observational studies. The

Conflict of interest

The authors do not have any conflict of interest with regard to the findings of the study.

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Annex 2: Amharic Survey Questionnaire

የመጠይቁ መለያ ቁጥር: _____

በደቡብ ክልል፣ ሲዳማ ዞን በሚገኙ ነፍሱ-ጡር እናቶች ላይ የምግብ እጥረት ደረጃና ተያያዥ ምክኒያቶችን ለማጥናት የተዘገጀ መጠይቅ

መረጃ መስጫ ቅጽ

ወድ ተሳታፊ፤

እንደምን አደሩ/ዎሉ። ከኔጋ ለመወያየት ፍቃደኛ በመሆኖ አመሰግናለሁ። እኔ _____ ስሆን በአካባቢያቸው በሚገኙ ነፍሱ-ጡር እናቶች ላይ የምግብ እጥረት ደረጃ፣ ተያያዥ ምክኒያቶችንና በሚወለደው ልጅ ክብደት ላይ ያለውን ተፅእኖ ለማጥናት የመጣዉ የጥናት ቡድን አባል ነኝ። ጥናቱ የሶስተኛ ዲግሪ ትምህርት አካል ሆኖ በአዲስ አበባ ዩኒቨርሲቲ ተማሪ የሚከናወን ነው።

በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከ 20-25 ዳቂቃ የሚወስድ ቃለ-መጠይቅ አደርጎለታለሁ። ክብደትና ቁመቶትንም እሰጣለሁ። ከዚህ በተጨማሪ ከእርሶ ከስድስት ሚሊ ያልበለጠ የደምና ናሙና እወስዳለሁ። የደም ናሙና ለመውሰድ ንጹህ መሳሪያዎችን ስለምጠቀም የበሽታ መተላለፍ አደጋ አይኖርብዎትም። ሆኖም ደም በምወስድበት ጊዜ አንስተኛ ህመም ሊሰማዎት ይችላል። ከእርሶ የወሰድኩት የደም ናሙና ለተለያዩ የምግብ አይነቶች እጥረት እንዳለብዎ ወይም እንደሌለብዎ ለማወቅ ከሚደረግ ምርምራ ወጪ ለሌላ ምርመራ ፈጽሞ አይወልደም። ይህ ምርምር እንደተጠናቀቀም የወሰድኩት የደም ናሙና በአግባቡ ይወገዳል። የዛሬ መጠይቁን ከጨረስኩ በኋላ ይህን እርግዝና እንደተገላገሉ በአካባቢዉ ያሉ የጤና ኤክስቴንሽንና የባህሪ ፍቃድ መልእክተኞች የልጅዎን ክብደትና ቁመት ይሰጣሉ።

በጥናቱ ተሳትፎዎ ወቅት የምግብ እጥረት አንዳለብዎት ከታወቀ አስፈላጊዉ የስነምግብ ትምህርት ወይም መድሀኒት ይሰጥዎታል። ሆኖም ተሳትፎዎን አስመልክቶ የሚደረግሎት የገንዘብ ክፍያ አይኖርም።

ስሞ ከማንኛዉም አይነት የጥናቱ ግኝት ጋር ተያይዞ አይቀርብም። በመጠይቁ ጊዜ ለመመለስ የማይፈልጉት ጥያቄ ካለ ያለመመለስ መብት ያሉት ሲሆን ጥናቱንም በፈለጉት ጊዜ ማቋረጥ ይችላሉ። ሆኖም የሚሰጡን እውነተኛ የተሟላ መልስና በጥናቱ ላይ የሚያደርጉት ተሳትፎ በአካባቢዉ ያለውን የምግብ እጥረት ደረጃ በደረጃ ለማሻሻል የራሱ የሆነ አስተዋጽኦ ስለሚኖረዉ ሙሉ ተሳትፎዎን አጥብቀን እንሻለን። በጥናቱ ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በእርሶ ፍላጎትና ምርጫ ላይ የተመሰረተ ነው። በጥናቱ አለመሳተፍ ቢመርጡ መንግስታዊ ወይም መንግስተዊ ካልሆኑ ድርጅቶች የሚያገኙትን ማንኛዉም የጤና ክብካቤ አይከለክሉም። ሌላም ማንኛዉም አይነት ተጽእኖ አይደርስብዎትም። የሚያጡትም ማንኛዉም ጥቅም አይኖርም።

ጥናቱ በሚከናወንበት ጊዜ ምንም አይነት ሀሳብ ቢገባዎት ጥናቱን አስቁመዉ ሊጠይቁኝ ይችላሉ። በዛሬዉ ጉብኝቱ ላይ ግልጽ ያልሆነሎት ነገር ካለ ይግለጹልኝና ላብራራ። ከዚህም በተጨማሪ ባስፈለጎት ጊዜ የዚህን ጥናት ዋና ተመራማሪ በ +251916822815 ወይምን ደግሞ ጥናቱን ያዕደቀዉን ኮሚቴ በ +251115538734 ደወለዉ በመጠየቅ ተጨማሪ መረጃ ማግኘት ይችላሉ።

መረጃ ሰብሳቢ፡ ማንኛዉንም ጥያቄ እንዲያነሱ ያበረታቷቸዉ።

የጥናት ተሳፍሮ ፍቃድ የመጠየቂያ ቅጽ

ከላይ የተሰጡትን መረጃዎች በማገናዘብና በመረዳት በጥናቱ ለመሳተፍ ፋቃደኛ ኖት?

አዎን

የተሳታፊዎ ስምና ፊርማ/የጣት አሻራ

ስም _____ ፊርማ/የጣት አሻራ _____ ቀን _____

(ወደ ቃለ-መጠይቁ ይለፉ)

የለም (ቃለ-መጠይቁን ያቁሙ)

የመረጃ ስብሰቢዉ ፊርማ

ስም _____ ፊርማ _____ ቀን _____ ሰዓት _____

የተቆጣጣሪዉ ፊርማ

ስም _____ ፊርማ _____ ቀን _____

1. የጥናቱን አካባቢ የተመለከቱ መሰረታዊ መረጃዎች			
ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0101	የወረዳው ስም	_____	
0102	የቀበሌው ስም	_____	
0103	የአካባቢው የአየር ጠባይ	1. ደጋ 2. ወይና ደጋ 3. ቆላ	
0104	የቀበሌው ክፍታ ከባህር ጠለል በላይ	_____ ሜትር	
2. ተሳታፊዎችን የተመለከቱ መሰረታዊ መረጃዎች			
ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0201	የተሳታፊዎች እድሜ (በተጠናቀቀ አመት)	_____ አመት	
0202	የተሳታፊዎች ብሄር	1. ሲዳማ 2. ወላይታ 3. አሮሞ 4. አማራ 5. ጌድኦ 6. ጉራጌ 7. ትግሬ 8. የተለየ ከሆነ ይጠቀስ _____	
0203	የተሳታፊዎች ሀይማኖት	1. ኦርቶዶክስ 2. ፕሮቴስታንት 3. ካቶሊክ 4. ሙስሊም 5. የተለየ ከሆነ ይጠቀስ _____	
0204	የስራ አይነት	1. የቤት እመቤት 2. አርሶ/አርብቶ አደር 3. የጉልበት ሰራተኛ 4. አንስተኛ ንግድ 5. የመንግስት ወይም መ.ያ.ድ. ተቀጣሪ 6. የተለየ ከሆነ ይጠቀስ _____	
0205	የትምህርት ደረጃ (በተጠናቀቀ የትምህርት አመት)	1. ያልተማሩ 2. መደበኛ ያልሆነ ት/ት የተማሩ (ማንበብ ወይም/እና መጻፍ የሚችሉ) 3. መደበኛ ትምህርት (_____ ክፍል ያጠናቀቁ) 4. ከፍተኛ ትምህርት የተማሩ	
0206	የጋብቻ ሁኔታ	1. ጨርሶ ያላገቡ 2. የተፋቱ/የተለያዩ 3. ባለቤታቸው የሞተባቸው 4. ያገቡ/አብረው የሚኖሩ	→ ወደ 0212
0207	የባለቤቶች እድሜ ምን ያህል ነው? (በተጠናቀቀ አመት)	1. _____ አመት 2. አላውቅም/አርግጠኛ አይደለሁም	

0208	ባለቤትዎ የምን ብሄር አባል ናቸው?	<ol style="list-style-type: none"> 1. ሲዳማ 2. ወላይታ 3. አሮሞ 4. አማራ 5. ጌድኦ 6. ጉራጌ 7. ትግሬ 8. የተለየ ከሆነ ይጠቀስ _____ 	
0209	ባለቤትዎ የምን ሀይማኖት ተከታይ ናቸው?	<ol style="list-style-type: none"> 1. ኦርቶዶክስ 2. ፕሮቴስታንት 3. ካቶሊክ 4. ሙስሊም 5. የተለየ ከሆነ ይጠቀስ _____ 	
0210	የባለቤቶች ስራ ምንድን ነው?	<ol style="list-style-type: none"> 1. አርሶ/አርብቶ አደር 2. የጉልበት ሰራተኛ 3. አንስተኛ ንግድ 4. የመንግስት ወይም መ.ያ.ድ. ተቀጣሪ 5. የተለየ ከሆነ ይጠቀስ _____ 	
0211	የባለቤቶች የትምህርት ደረጃ ምን ያህል ነው?	<ol style="list-style-type: none"> 1. ያልተማሩ 2. መደበኛ ያልሆነ ት/ት የተማሩ (ማንበብ ወይም/እና መጻፍ የሚችሉ) 3. መደበኛ ትምህርት (____ ክፍል ያጠናቀቁ) 4. ከፍተኛ ትምህርት የተማሩ 5. አላወቅም/አርግጠኛ አይደለሁም 	
0212	በአብዛኛው ጊዜ ቤተሰቦች የሚጠቀሙበት የወ.ሃ መገኛ ምንድን ነው?	<ol style="list-style-type: none"> 1. የግል ወይም የጋራ የቧንቧ ወ.ሃ 2. የተጠበቀ የወ.ሃ ጉድጓድ/ምንጭ 3. ያልተጠበቀ የወ.ሃ ጉድጓድ/ምንጭ 4. ወራጅ ወንዝ/ኩራ/ሀይቅ 5. የተለየ ከሆነ ይጠቀስ _____ 	
0213	ቤተሰቦች የራሱ የሆነ መጻጻጃ ቤት አለው?	<ol style="list-style-type: none"> 1. አዎን 2. የለም 	
0214	የቤተሰብ ቁጥር (መረጃ ስብሰባ: ቋሚ የቤተሰብ ነዋሪዎችን ብቻ ይመለከታል።)	_____	

3. የቤተሰቡን ሀብትና ንብረት የተመለከቱ መረጃዎች			
ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0301	የራስዎት የሆነ የገቢ ምንጭ አሎት?	1. አዎን 2. የለም _____	→ ወደ 0303
0302	መልሶ አዎን ከሆነ የወር ገቢዎት ምን ያህል ይሆናል?	_____ ብር	
0303	በአጠቃላይ የቤተሰብ የወር ገቢ መጠን ምን ያህል ይሆናል? (መረጃ ሰብሳቢ፡ የተለያዩ የገቢ ምንጮችን እንዲያስታወሱ ይርዷቸዋል።)	_____ ብር	
0304	ቤተሰብ ለእርሻ የሚሆን (የይዘታ ወይም የክራይ) መሬት አለውን?	1. አዎን 2. የለም _____	→ ወደ 0306
0305	የመሬቱ መጠን በአካባቢው የልኬት ስያሜ ምን ያህል ነው?	1. _____ መጠን. መረጃ ሰብሳቢ፡ የመለኪያውን አይነት ከዚህ ይጻፍ (_____) 2. አላውቅም/እርግጠኛ አይደለሁም	
0306	ቤተሰብ ከሚከተሉት ውስጥ የትኞቹን የቤት እንስሳት ባለቤት ነው? ቁጥራቸውን ይጥቀሱ። የወተት ላም ወይም በሬ? ፈረስ፣ አህያ ወይም በቅሎ? ፍየል ወይም በግ? ዶሮ ወይም ጫጩት?	<u>ቁጥር</u> _____ _____ _____	
0307	ከሚከተሉት መካከል ይትኞቹን ከቤተሰብ አንዱ አባል አለው/ላት ወይም በቤትዎ ይገኛል?		
ሀ	ፋኖስ?	1. አዎን	2. የለም
ለ	አልጋ?	1. አዎን	2. የለም
ሐ	ወንበር?	1. አዎን	2. የለም
መ	ጠረጴዛ?	1. አዎን	2. የለም
ሰ	ሬዲዮ?	1. አዎን	2. የለም
ረ	የኤለክትሮክ ምጣድ?	1. አዎን	2. የለም
ሠ	ሳይክል?	1. አዎን	2. የለም
ሸ	ሞባይል ስልክ?	1. አዎን	2. የለም
ቀ	ስልክ (ሞባይል ያልሆነ)?	1. አዎን	2. የለም
በ	ጋሪ?	1. አዎን	2. የለም
ተ	የሞተር ሳይክል?	1. አዎን	2. የለም
ቸ	ቴሌቭዥን?	1. አዎን	2. የለም
ነ	ጀልባ?	1. አዎን	2. የለም
ኘ	ወፍጮ?	1. አዎን	2. የለም
0308	ቤተሰብ የኤሌክትሪክ አገልግሎት ተጠቃሚ ነውን?	1. አዎን	2. የለም
0309	የመኖሪያ ቤትዎ መስኮት አለውን?	1. አዎን	2. የለም

0310	ቤተሰቦች የሚኖርበት ቤት ወለል የተሰራው ከምንድን ነው?	1. አፈር 2. እንጨት፣ ጣዉላ ወይም ቀርከሃ 3. ያለቀለት ወለል (ሲሚንቶ፣ ጡብ ወዘተ) 4. የተለየ ከሆነ ይጠቀስ _____	
0311	ቤተሰቦች የሚኖርበት ቤት ጣራ የተሰራው ከምንድን ነው?	1. ቅጠል ወይም ሳር 2. ፕላስቲክ ወይም ላስቲክ 3. ቆርቆሮ 4. የተለየ ከሆነ ይጠቀስ _____	
0312	ቤተሰቦች የሚኖርበት ቤት ግድግዳ የተሰራው ከምንድን ነው?	1. እንጨት፣ ጣዉላ ወይም ቀርከሃ 2. በጭቃና ድንጋይ 3. በጭቃና እንጨት 4. ጡብ፣ ብሎኬት ወይም ሲሚንቶ 5. የተለየ ከሆነ ይጠቀስ _____	
0313	ከቤተሰቦች አባል ውስጥ አንዱ በባንክ ወይም በጥቃቅን የብድር ተቀዋሚት ውስጥ ተቀማጭ ብር አለው/አላት?	1. አዎን 2. የለም 3. አላውቅም/እርግጠኛ አይደለሁም	
0314	ቤተሰቦ በአብዛኛው ጊዜ ምግብ ለማዘጋጀት ምን አይነት የሃይል ምንጭ ይጠቀማል?	1. አንጨት 2. ከሰል 3. ከብት/ፍግ 4. ነጭ ጋዝ/ላንባ 5. የተለየ ከሆነ ይጠቀስ _____	

4. ስነ-ተዋልዶን የተመለከቱ መረጃዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0401	የመጨረሻውን ያልተዛባ የወር አበባ ያዩበት የመጀመሪያ ቀን መቼ ነበር?	1. ___/___/___ (ቀ/ወ/ዓም) 2. አላስታወስም/እርግጠኛ አይደለሁም	
0402	የስንት ወር ነፍሱጡር ኖት?	1. _____ ወራት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0403	ከዚህ በፊት ልጅ ወልደው ያወቃሉ?	1. አዎን 2. የለም _____	ወደ 0407
0404	የመጀመሪያ ልጅን ሲወልዱ ስንት አመቶት ነበር?	1. _____ አመት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0405	የመጨረሻ ልጅን የወለዱት ከስንት ወራት በፊት ነበር?	1. ከ _____ ወራት በፊት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0406	እስከ አሁን በጠቅላላው ስንት ልጆች ወልደዋል? (መረጃ ሰብሳቢ፡ በህይወት የተወለዱትን ልጆች ብቻ ይመዝግቡ)	_____ ልጆች	
0407	ወርጃ ወይም የሽል መሞት ገጥሞት ያወቃል? (መረጃ ሰብሳቢ፡ ሆን ተብሎ የተደረገ ወይም ሆን ተብሎ ያልተደረገ ወርጃ ይጨምራል)	1. አዎን 2. የለም _____	ወደ 0410
0408	መልሶት አዎን ከሆነ ምን ያህል?	_____ ወርጃ/የሽል መሞት	
0409	ወርጃ ወይም የሽል መሞት ለመጨረሻ ጊዜ የገጠሞት መቼ ነበር?	1. ከ _____ ወራት በፊት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0410	የመጀመሪያ የወር አበባዎትን በስንት አመቶት አዩ?	1. _____ አመት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0411	ለመጀመሪያ ጊዜ ሲያገቡ ስንት አመቶት ነበር?	1. _____ አመት 2. አላስታወስም/እርግጠኛ አይደለሁም	

5. የቤተሰብ ምጣኔን የተመለከቱ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ						
0501	ዘመናዊ የቤተሰብ መቆጣጠሪያ ተጠቅመዋል ያወቃሉ?	1. አዎን 2. የለም _____	→ ወደ 0601						
0502	መልሶት አዎን ከሆነ ምን ተጠቀሙ?	1. _____ 2. _____ 3. _____ 4. _____							
0503	ዘመናዊ የቤተሰብ መቆጣጠሪያ በባለፈው 5 አመት ተጠቅመዋል ያወቃሉ?	1. አዎን 2. የለም _____	→ ወደ 0601						
0504	መልሶት አዎን ከሆነ ከሚከተሉት ውስጥ የትኛውንና ለምን ያህል ጊዜ እንደተጠቀሙ ቢገልጹልን?	<table border="1"> <tr> <td>የሚጥጥ እንክብል</td> <td>ለ _____ ወራት</td> </tr> <tr> <td>በመርፌ የሚሰጥ</td> <td>ለ _____ ወራት</td> </tr> <tr> <td>በክንድ የሚቀበር</td> <td>ለ _____ ወራት</td> </tr> </table>	የሚጥጥ እንክብል	ለ _____ ወራት	በመርፌ የሚሰጥ	ለ _____ ወራት	በክንድ የሚቀበር	ለ _____ ወራት	
የሚጥጥ እንክብል	ለ _____ ወራት								
በመርፌ የሚሰጥ	ለ _____ ወራት								
በክንድ የሚቀበር	ለ _____ ወራት								
0505	ከላይ የተጠቀሱትን የቤተሰብ ምጣኔ መንገዶች መጠቀም የጀመሩት በምን ያህል እድሜዎት ነበር?	1. በ _____ አመት 2. ከላይ የተጠቀሱትን አልተጠቀምኩም 3. አላስታወስም/እርግጠኛ አይደለሁም							

6. ጡት ማጥባትን የተመለከቱ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0601	ጡት አጥብተዋል ያወቃሉ?	1. አዎን 2. የለም _____	→ ወደ 0701
0602	በባለፈው አምስት አመት ጡት አጥብተዋል ያወቃሉ?	1. አዎን 2. የለም _____	→ ወደ 0701
0603	በቀርብ ጊዜ ላጠቡት ልጅ ለምን ያህል ጊዜ የጡት ወተት ብቻ (ያለተጨማሪ ምግብ) ሰጡ?	1. _____ ወራት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0604	በቀርብ ጊዜ ላጠቡት ልጅ በአጠቃላይ ለምን ያህል ጊዜ ጡት አጠቡ?	1. _____ ወራት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0605	በቀርብ ጊዜ ላጠቡት ልጅ ከዚህ በታች በተጠቀሱት እድሜዎቹ/ቿ በአማካኝ በቀን ለምን ያህል ጊዜ ያጠቡ ነበር?		
	0-5 ወራት	1. _____ ጊዜ/በቀን 2. አላስታወስም/እርግጠኛ አይደለሁም 3. የተለየ ከሆነ ይጠቀስ _____	
	6-11 ወራት	1. _____ ጊዜ/በቀን 2. አላስታወስም/እርግጠኛ አይደለሁም 3. የተለየ ከሆነ ይጠቀስ _____	
	12-23 ወራት	1. _____ ጊዜ/በቀን 2. አላስታወስም/እርግጠኛ አይደለሁም 3. የተለየ ከሆነ ይጠቀስ _____	
0606	በቅርቡ ጡት ባጠቡበት ጊዜ አመጋገቦት ከወትሮው ጋር ሲወዳደር እንዴት ነበር?	1. ከተለመደው በታች 2. እንደተለመደው 3. ከተለመደው በላይ 4. አላስታወስም/እርግጠኛ አይደለሁም	
0607	በቅርቡ ጡት በጠቡበት ጊዜ የቫይታሚን ኤ እንክብል ተሰጥቶት ነበር? (መረጃ ሰብሳቢ: የቫይታሚን ኤ እንክብል ያላዩዎቸው።)	1. አዎን 2. የለም 3. አላስታወስም/እርግጠኛ አይደለሁም	

7. ሀመምና የጤና አገልግሎት የተመለከቱ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0701	ከቤቶ ቅርብ ወደሚገኘው የጤና ተቋም በእግር ለመሄድ ምን ያህል ጊዜ ይወስዳል? (መረጃ ሰብሳቢ: ስለ ደርሶ መልስ ርቀት ይጠይቋቸው።)	1. _____ ደቂቃ 2. አላስታወስም/እርግጠኛ አይደለሁም	
0702	በዚህ የእርግዝና ወቅት የእርግዝና ክትትል አድርገው ነበርን?	1. አዎን 2. የለም _____	ወደ 0710
0703	መልሶት አዎን ከሆነ የእርግዝና ክትትል ከየት ወሰዱ/አየወሰዱ ነው?	1. ጤና ኬላ 2. ክሊኒክ 3. ጤና ጣቢያ 4. ሆስፒታል 5. የሰለጠኙ የልምድ አዋላጅ/የጤና ተጠሪ 6. የተለየ ከሆነ ይጠቀስ _____	
0704	በዚህ እርግዝና ለመጀመሪያ ጊዜ የእንርግዝና ክትትል ሲያደርጉ የስንት ወር እርጉዝ ነበሩ?	1. _____ ወራት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0705	በአጠቃላይ በዚህ እርግዝና ለምን ያህል ጊዜ የእርግዝና ክትትል አደረጉ?	1. _____ ጊዜ 2. አላስታወስም/እርግጠኛ አይደለሁም	
0706	በዚህ እርግዝና ጊዜ ባደረጉት ክትትል ከሚከተሉት የትኞቹ ቢያንስ አንዴ ተደርጎታል?		
	ቁመት መለካት?	1. አዎን 2. የለም 3. አላስታወስም/እርግጠኛ አይደለሁም	
	ክብደት መመዘን?	1. አዎን 2. የለም 3. አላስታወስም/እርግጠኛ አይደለሁም	
0707	በዚህ እርግዝና የአይረን/ፎሌት እንክብል ተሰጥቶት ነበርን? (መረጃ ሰብሳቢ: በአካባቢው በብዛት የሚገኙ የአይረን/ፎሌት እንክብል አይነቶች ያሳይዎቸው።)	1. አዎን 2. የለም _____ 3. አላስታወስም/እርግጠኛ አይደለሁም _____	ወደ 0710 ወደ 0710
0708	መልሶት አዎን ከሆነ እንክብሉን መውሰድ የጀመሩት ከስንት ሳምንታት በፊት ነበር?	1. ከ _____ ሰምንታት በፊት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0709	በአጠቃላይ በባለፉት 4 ሳምንታት ምን ያህል የአይረን/ፎሌት እንክብል ወሰዱ?	1. _____ እንክብል 2. አላስታወስም/እርግጠኛ አይደለሁም	
0710	በዚህ እርግዝና ከማህፀን ደም መፍሰስ (በወር አበባ ከተለመደው በላይ) ገጥሞት ነበርን?	1. አዎን 2. የለም	
0711	በዚህ እርግዝና ከአንድ ቀን በላይ የቆየ ትኩሳት ገጥሞት ነበርን?	1. አዎን 2. የለም _____	ወደ 0714
0712	መልሶት አዎን ከሆነ ዘመናዊ ህክምና አግኝተው ነበርን?	1. አዎን 2. የለም _____	ወደ 0714
0713	መልሶት አዎን ከሆነ የትኩሳቱ መነሻ ምንድን ነው ተብሎ ከሀኪም ተገለፀሎ?	1. _____ 2. አልተነገረኝም/አላስታወስም	
0714	በባለፈው ስድስት ወራት በወባ በሽታ (በሀኪም የተረጋገጠ) ተጠቅተው ነበርን?	1. አዎን 2. የለም 3. አላስታወስም/እርግጠኛ አይደለሁም	
0715	በዚህ እርግዝና ከባድ የራስ ምታት፣ አጠቃላይ የሰውነት ማበጥና አይን ላይ ብገር የማለት ስሜት ተሰምቶት ያወቃል?	1. አዎን 2. የለም	

0716	በዚህ እርግዝና በቀን ለማየት የመቸገር ሁኔታ ገጥሞት ነበርን?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
0717	በዚህ እርግዝና በምሽት ለማየት የመቸገር ሁኔታ (ዳፍንት) ገጥሞት ነበርን? (መረጃ ሰብሳቢ: በአካባቢው በስፋት የሚታወቀውን የዳፍንት መጠሪያ ተጠቅመው ይጠይቁ።)	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
0718	በባለፈው እንደ ሳምንት ከሁለት ቀን በላይ የቆየና በቀን ሶስት ወይም ከሶስት ጊዜ በላይ የሚከሰት ትውክት ገጥሞት ነበርን?	1. አዎን 2. የለም	
0719	በሀኪም የተረጋገጠ ማንኛውም በሽታ አለቦትን?	1. አዎን (ይግለፁ) _____ 2. የለም	
0720	በአሁኑ ሰዓት ማንኛውም መድሃኒት እየወሰዱ ነው?	1. አዎን (ይግለፁ) _____ 2. አዎን የመድሃኒቱን ስም አላወቅም 3. የለም	
0721	በዚህ እርግዝና ማንኛው የስነ-ምግብ ትምህርት ወይም ምክር አግኝተው ነበርን?	1. አዎን 2. የለም _____	ወደ 0801
0722	መልሶት አዎን ከሆነ የስነ-ምግብ ትምህርት ወይም ምክር የሰጡት ማን ነበር? (መረጃ ሰብሳቢ: ከአንድ በላይ መልስ ሊኖር ይችላል።)	1. የጤና ኤክስፔንሽን ሰራተኛ 2. የጤና በጎ ፍቃድ መልክተኞች 3. ሌሎች የጤና ባለሙያዎች 4. አላስታወስም/እርግጠኛ አይደለሁም 5. የተለየ ከሆነ ይጠቀስ _____	

8. የምግብ አመጋገብን የተመለከቱ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
0801	በአካባቢዎ በአብዛኛው ጊዜ የሚበላ ዋና የምግብ አይነት ምንድን ነው?	1. በእንሰት ላይ የተመሰረቱ ምግቦች 2. በእህሎች ላይ የተመሰረቱ ምግቦች 3. የተለየ ከሆነ ይጠቀስ _____	
0802	በአካባቢዎ በአብዛኛው ጊዜ ጥቅም ላይ የሚውል እህል የትኛው ነው? (መረጃ ሰብሳቢ: ከአንድ በላይ መልስ ሊኖር ይችላል።)	1. በቆሎ 2. ማሽላ 3. ጤፍ 4. ስንዴ 5. ገብስ 6. ዳጉሳ 7. የተለየ ከሆነ ይጠቀስ _____	
0803	በአካባቢዎ በአብዛኛው ጊዜ ጥቅም ላይ የሚውል ጥራጥሬ የትኛው ነው? (መረጃ ሰብሳቢ: ከአንድ በላይ መልስ ሊኖር ይችላል።)	1. ባቁላ 2. አተር 3. ሽንብራ 4. ቦሎቄ 5. አደንንጫ 6. የተለየ ከሆነ ይጠቀስ _____	

0804	በአብዛኛው ጊዜ በቤተሰቦ እርሻ መሬት ላይ የሚለሙ ሰብሎችን ይዘርዝሩ።	1. _____ 2. _____ 3. _____ 4. _____	
0805	በዚህ እርግጠና ያሉትን የምግብ አወሳሰድ አወትሮው ጋር ሲወዳደር እንዴት ይመዘኑታል?	1. ከተለመደው በታች 2. እንደተለመደው 3. ከተለመደው በላይ	
0806	በአሁኑ ጊዜ ከማንኛውም መንግሥታዊ ሆነ መ.ያ.ድ. የምግብ እርዳታ እያገኙ ነው?	1. አዎን 2. የለም _____	→ ወደ 0809
0807	ምን አይነት የምግብ እርዳታ እያገኙ ነው? (መረጃ ሰብሳቢ፡ ከአንድ በላይ መልስ ሊኖር ይችላል።)	1. ስንዴ 2. በቆሎ 3. ጥራጥሬ 4. ዘይት 5. አልሚ ምግብ 6. የተለየ ከሆነ ይጠቀስ _____	
0808	በቅርብ ጊዜ ወስጥ የምግብ እርዳታ የተቀበሉት መቼ ነው?	1. ከ _____ ሳምንታት በፊት 2. አላስታወስም/እርግጠኛ አይደለሁም	
0809	አትክልትና ፍራፍሬ የሚያለሙበት የጓሮ እርሻ አሎት?	1. አዎን 2. የለም _____	→ ወደ 0811
0810	መልሶት አዎን ከሆነ በአሁኑ ሰአት ምን አይነት አትክልትና ፍራፍሬ አልምተዋል?	1. _____ 2. _____ 3. _____ 4. _____	
0811	አሁን ደግሞ በባለፈው 24 ሰአት (ከትላንት ንጋት እስከ ዛሬ ንጋት ድረስ) ስለተመገቧቸው ምግብ አይነቶች እጠይቁታለሁ። የሚከተሉትን ምግቦች ተመግበው ነበርን?		
ሀ	ከበቆሎ፣ ስንዴ፣ ጤፍ፣ ዘንጋዳ፣ ማሽላ፣ ወዘተ የተሰራ ምግብ (ዳቦ፣ እንጆራና የመሳሰሉትን ጨምሮ)?	1. አዎን 2. የለም	
ለ	ከሽንብራ፣ አተር፣ ምስር፣ ባቁላ፣ አደንጓሬ፣ ቦሎቄ ወዘተ ካሉ የተሰራ ምግብ (ወጥ፣ ቆሎ፣ ንፍሮ የመሳሰሉትን ጨምሮ)?	1. አዎን 2. የለም	
ሐ	እንደ ድንች፣ ካሳቫ፣ እንሰት፣ ቦይና፣ ጎደሬ ካሉ ስራስሮች የተሰራ ምግብ?	1. አዎን 2. የለም	
መ	እንደ ካሮት፣ ቀይስር፣ ዝኩኒ፣ ማንጎ፣ ፓፓያ፣ ከመሳሰሉ አትክልትና ፍራፍሬዎች ወይም እንደ ጎመንና ቆስጣ ያሉ አረንጓዴ ቅጠላ ቅጠሎች የተሰራ ምግብ?	1. አዎን 2. የለም	
ሰ	ሌላ ፍራፍሬ?	1. አዎን 2. የለም	
ረ	ሌላ አትክልት?	1. አዎን 2. የለም	
ሠ	ከማንኛውም አይነት ስጋ የተሰራ ምግብ?	1. አዎን 2. የለም	
ሸ	ከማንኛውም ወተትና የወተት ወጤቶች የተሰራ ምግብ?	1. አዎን 2. የለም	

ቀ	ከእንቁላል የተሰራ ምግብ?	1. አዎን 2. የለም	
በ	ከአሳ የተሰራ ምግብ?	1. አዎን 2. የለም	
ተ	ከዘይት፣ ቅቤ ወይም ስብ የተሰራ ምግብ?	1. አዎን 2. የለም	
ቸ	ስኳርና ማር?	1. አዎን 2. የለም	
ነ	ሌሎች ምግቦች?	1. አዎን 2. የለም	
0812	በባለፈው 24 ሰዓት (ከትላንት ንጋት እስከ ዛሬ ንጋት ድረስ) መክሰስን ጨምሮ ምን ያህል ጊዜ ምግብ ተመገቡ?	1. _____ ጊዜ 2. አላስታወስም/እርግጠኛ አይደለሁም	
0813	በአማካኝ በአንድ ቀን ውስጥ ምን ያህል ሻይ ይጠጣሉ? (መረጃ ሰብሳቢ፡ መጠኑን በ70 ሚ.ሊ. መካከለኛው የቡና ስኒ ይተምኑ።)	1. _____ ስኒ 2. ሻይ በአብዛኛው ጊዜ አልጠጣም 3. አላስታወስም/እርግጠኛ አይደለሁም	
0814	በአማካኝ በአንድ ቀን ውስጥ ምን ያህል ቡና ይጠጣሉ? (መረጃ ሰብሳቢ፡ መጠኑን በ70 ሚ.ሊ. መካከለኛው የቡና ስኒ ይተምኑ።)	1. _____ ስኒ 2. ቡና በአብዛኛው አልጠጣም 3. አላስታወስም/እርግጠኛ አይደለሁም	
0815	በአማካኝ በአንድ ሳምንት ውስጥ ምን ያህል የአልኮል መጠጦች ይጠጣሉ? (መረጃ ሰብሳቢ፡ መጠኑን በ70 ሚ.ሊ. መካከለኛው የቡና ስኒ ይተምኑ። በአከባቢው የሚዘወተሩትን ባህላዊ የአልኮል አይነቶች ያስታውሷቸዋል።)	1. _____ ስኒ 2. አልኮል በአብዛኛው አልጠጣም 3. አላስታወስም/እርግጠኛ አይደለሁም	
0816	መረጃ ሰብሳቢ፡ አንድ ማንኪያ የገበታ ጨው በመውሰድ የአዮዲን መጠኑን ይለኩ።	1. አዮዲን የለውም 2. ከ 15 ፒ.ፒ.ኤም በታች 3. ከ 15 ፒ.ፒ.ኤም በላይ	
0817	በአከባቢዎት በተለምዶ፣ በባህል ወይም በሀይማኖት ምክኒያት ነፍሱ-ጡሮች እንዳይበሉት የሚከለክል ምግብ አለን?	1. አዎን 2. የለም _____ → ወደ 0821 3. አላስታወስም/እርግጠኛ አይደለሁም _____ → ወደ 0821	
0818	መልሶት አዎን ከሆነ የምግብ አይነቶችን ይዘርዝሩ?	1. _____ 2. _____ 3. _____	
0819	በዚህ እርግዝና ወቅት ማንኛውንም ምግብ በባህል/ሀይማኖት ምክኒያት እንዳይወስዱ ተከልክለው (ወይም ታቅበው) ነበርን?	1. አዎን 2. የለም _____ → ወደ 0821	
0820	መልሶት አዎን ከሆነ የምግብ አይነቶችን ይዘርዝሩ?	1. _____ 2. _____ 3. _____ 4. _____	

0821	አሁን ቀጥለው የተዘረዘሩት የምግብ አይነቶች በባለፉት አንድ ሳምንት (ሰባት ቀን) ምን ያህል ጊዜ ተመግበዎቸው እንደነበር እጠይቃለሁ።		
ሀ	አማንኛውም አይነት ስጋ የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ለ	አማንኛውም ወተትና የወተት ወጤቶች የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሐ	ከእንቁላል የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
መ	ከአሳ የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሰ	እንሰትን መሰራት ካደረጉ (እንደ ቆጮ፣ ቁንጨሳ፣ ቡላ ወዘተ) የመሳሰሉ ምግቦች?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ረ	እንደ ድንች፣ ካሳቫ፣ ቦይና፣ ጎዶሬ ካሉ ስራስሮች የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሠ	ከቆሎ፣ ስንዴ፣ ጤፍ፣ ዘንጋዳ፣ ማሽላ፣ ወዘተ የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሸ	ከሽንብራ፣ አተር፣ ምስር፣ ባቁላ፣ አደንጌሬ፣ ቦሎቄ ወዘተ ካሉ የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ቀ	እንደ ካሮት፣ ቀይስር፣ ዝኩኒ፣ ማንጎ፣ ፓፓያ፣ ከመሳሰሉ አትክልትና ፍራፍሬዎች ወይም እንደ ጎመንና ቆስጣ ያሉ አረንጓዴ ቅጠላ ቅጠሎች የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
በ	ሌላ ፍራፍሬ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ተ	ሌላ አትክልት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ቸ	ከዘይት፣ ቅቤ ወይም ስብ የተሰራ ምግብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
0822	ለመጨረሻ ጊዜ ምግብ የተመገቡት ከስንት ሰዓታት በፊት ነበር?	_____ ሰዓታት	
0823	ምግብ ለቤተሰብ በሚዘጋጅበት ጊዜ የሚከተሉትን በአብዛኛው በቤትዎ ያከናውናሉን?		
ሀ	የእህል/ጥራጥሬዎችን የላይ ሽፋን በመውቃት፣ በመፍጨት፣ በማሸት ወዘተ ማስወገድ?	1. አዎን	
		2. የለም	
ለ	የእህል/ጥራጥሬዎችን ዱቄት አቡክቶ ለቀናት እስኪቆመጥ/ከፍ እስኪል ማቆየት?	1. አዎን	
		2. የለም	
ሐ	እንሰትን በመቅበር/በማቆየት እንዲቆመጥ ማድረግ?	1. አዎን	
		2. የለም	
መ	እህል/ጥራጥሬዎችን ለሰአታት በውሃ መዘፍዘፍ?	1. አዎን	
		2. የለም	
ሰ	እህል/ጥራጥሬዎችን ወደበቆልት መቀየር?	1. አዎን	
		2. የለም	
9. የስራ ጫናን የተመለከቱ ጥያቄዎች			
ተ.ቁ	ጥያቄዎች	ምርጫዎች	
ማስታወሻ			
0901	በባለፈው አንድ ሳምንት ውስጥ የሚከተሉት ስራዎች ምን ያህል ጊዜ አከናውነዋል?		
ሀ	ከሰላሳ ደቂቃ በላይ የእግር መንገድ በመጓዝ ወሃ መቅዳት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ለ	ከሰላሳ ደቂቃ በላይ የእግር መንገድ በመጓዝ ማገደ/ከብት መልቀም?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሐ	ልብስ ማጠብ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
መ	ጉልጓሎ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ሰ	መቆፈ ወይም ማረስ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም
ረ	ማረም?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም

ሠ	ማጨድ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
ሸ	መወቃት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
ቀ	ጥራጥራ መፍጨት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
በ	መወቀጥ?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
ተ	እንስት ማዘጋጀት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
ቸ	እርከን መስራት?	1. _____ ጊዜ	2. እርግጠኛ አይደለሁም	
0902	በዚህ እርግዝና ጊዜ ያሎትን የስራ ጫና ከወትሮው ጋር ሲወዳደር እንዴት ይገልፁታል?	1. ከተለመደው በታች 2. እንደተለመደው 3. ከተለመደው በላይ		

10. የቤተሰብ ምግብ ዋስትናን የተመለከቱ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርጫዎች	ማስታወሻ
1001	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል የምትፈልጉትን (የምትመርጡትን) የምግብ አይነት በእጦት ምክኒያት ሳትመገቡ ቀርታችሁታል?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1002	በባለፈው አራት ሳምንታት ቤተሰቡ በቂ ምግብ ላያገኝ ይችላል ብለው ተጨንቀው ያወቃሉን?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1003	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት በአይነት የተወሰነ የምግብ አይነት ብቻ ተመግባቸዋል ነበር?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1004	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት ለወትሮው ልትመገቧቸው የማትፈልጉትን ምግቦች ተመግባቸዋል ነበር?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1005	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት በመጠኑ በቂ ነው ብለው የማያስቡትን ምግብ ተመገበው ነበር?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1006	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት በቀን ውስጥ በቁጥሩ በቂ ነው ብለው የማያስቡትን ምግብ ተመገበው ነበር?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1007	በባለፈው አራት ሳምንታት ውስጥ በቤትዎ ውስጥ በእጦት ምክኒያት ምንም የሚበላ ምግብ ያልነበረበት ሁኔታ ነበር?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1008	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት ተርባቸዋል አድራቸዋል ታወቃላቸዋል?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	
1009	በባለፈው አራት ሳምንታት ውስጥ እርሶ ወይም የቤተሰብ አባል በእጦት ምክኒያት ተርባቸዋል ወላቸዎች አድራቸዋል ታወቃላቸዋል?	1. አዎን 2. የለም 3. እርግጠኛ አይደለሁም	

ለተሳትፎዎ አመሰግናለሁ!!

Format to be filled by the data collector after physical examination of the woman

1. Body Height _____ cms
2. Body Weight _____ kgs
3. Middle Upper Arm Circumference _____ cms
4. Fundal Height _____ weeks
5. Goiter Present _____ Absent _____
6. If present the stage _____ stage
7. Pallor Present _____ Absent _____
8. Bitot's spot Present _____ Absent _____
9. Blood pressure _____

Checklist format to be filled by the lab technician after lab analysis

1. Hemoglobin level _____ gm/l
2. Serum zinc level _____ mg/l
3. Serum retinol level _____ μ mol/l

Annex 3: English Survey Questionnaire

Questionnaire identification number: _____

Level, correlates and effects on birthweight of prenatal zinc and vitamin A deficiencies in rural Sidama, SNNPR, Ethiopia.

Subject information sheet

Dear respondent

Good morning/Good afternoon. Thank you for your interest in talking with me today. I am _____ who is a member of a team conducting a study to assess the magnitude, determinants and effects on birthweight of malnutrition among pregnant women in your locality. The study is carried out as part of a PhD research conducted under the School of Public Health of Addis Ababa University.

The purpose of my visit today is to take information from you on the aforementioned topic. If you are willing to participate in the study, I will ask you questions lasting for about 20-25 minutes. I will also measure your weight and height so as to assess your nutritional status. In addition, I will take about 6 ml of venous blood sample. The blood will be drawn using sterile needle and syringe so that it will pose no risk of disease transmission; however, you may feel slight needle injection pain. The blood sample collected will only be analyzed for nutrients that the study is focused on and it will not be used for any different purpose. It will also be discarded after the end of the study. Upon the time of your birth, local health extension workers will visit you to measure the weight of your baby. In the study if you are found to have nutritional deficiencies, appropriate medication and nutrition education will be given. However, no financial payment will be made for your participation.

Your name will never be used in connection with any of your information that you will provide. You do not have to answer any question that you do not feel comfortable with, and you may end this task any time you want to. However, your honest answers to these questions and your continuous interest to participate study will help us in understanding of the situation of maternal malnutrition in your locality better, and will eventually help in designing and implementing appropriate intervention programs to alleviate the problem. Hence we would greatly appreciate your help in taking part in the study. Your participation is fully based on your interest and choice. Your participation or non participation will not be related with the health service that you will get from governmental and non governmental institutions.

If you have any unclarity on my visit you can ask me now so that I will elaborate it. During my stay if you come across with any concern, you can stop me and raise it anytime you want to. It is also possible to contact with the principal investigator through or the institutional review board which approved the study through the telephone address of +251916822815 or +251115538734, respectively.

Data collector: Please encourage the respondent to raise questions.

Informed Consent Form

With due understanding of the aforementioned information, are you willing to participate in the study?

Yes

Signature/finger print of the participant

Name _____ Signature/finger print _____ date _____

(Proceed with the interview)

No (Terminate the interview)

Signature of the interviewer

Name _____ Signature _____ date _____

Supervisors/Researcher remark and signature

Name _____ Signature _____ date _____

1. Basic Information about the Study Locality			
No	Questions	Choices	Remark
0101	Name of the woreda	_____	
0102	Name of the kebele	_____	
0103	Agro-ecological zone	1. Highlands 2. Midlands 3. Lowlands	
0104	Altitude of the kebele above sea level	_____ meters	
2. Socio-demographic Information			
No	Questions	Choices	Remark
0201	How old were you on your last birthday? (In completed years)	_____ Years	
0202	To which ethnic group do you belong?	1. Sidama 2. Woliyta 3. Oromo 4. Amhara 5. Gedio 6. Gurage 7. Tigray 8. If other specify _____	
0203	To which religion group do you belong?	1. Orthodox 2. Protestant 3. Catholic 4. Muslim 5. If other specify _____	
0204	What is your occupation/livelihood?	1. House wife 2. Farmer 3. Daily laborer 4. Petty trade 5. Privately owned business 6. Government or NGO employee 7. If other specify _____	
0205	What is your maximum level of education (in completed years of education)?	1. Illiterate 2. Information education (Can read or/and write) 3. Formal education (___ completed years of education) 4. Tertiary level of education	
0206	What is your current marital status?	1. Never married 2. Divorced/separated 3. Widowed 4. Married/living together	Q 0212
0207	How old is your husband/partner? (In completed years)	1. _____ years 2. I don't know/Not sure	

0208	To which ethnic group does your husband/partner belongs?	<ol style="list-style-type: none"> 1. Sidama 2. Woliyta 3. Oromo 4. Amhara 5. Gedio 6. Gurage 7. Tigray 8. If other specify _____ 	
0209	To which religious group does your husband/partner belongs?	<ol style="list-style-type: none"> 1. Orthodox 2. Protestant 3. Catholic 4. Muslim 5. If other specify _____ 	
0210	What is the usual occupation of your husband/partner?	<ol style="list-style-type: none"> 1. Farmer 2. Daily laborer 3. Petty trade 4. Government or NGO employee 5. If other specify _____ 	
0211	What is the educational status of your husband/partner (in completed years of education)?	<ol style="list-style-type: none"> 1. Illiterate 2. Information education (Can read or/and write) 3. Formal education (____ completed years of education) 4. Tertiary level of education 5. I don't know/Not sure 	
0212	What is the usual water source of your household?	<ol style="list-style-type: none"> 1. Tap water 2. Protected well or spring 3. Unprotected well or spring 4. Surface water 5. If other specify _____ 	
0213	Does your household own a toilet facility?	<ol style="list-style-type: none"> 1. Yes 2. No 	
0214	Household size (including all permanent residents)	_____	

3. Economic Information

No	Questions	Choices	Remark
0301	Do you have your own income?	1. Yes 2. No _____	Q 0303
0302	If Yes, how much do you earn in a typical month?	_____ Birr	
0303	In total how much is the monthly income of your household? <i>(DC: Please help her to remember different sources of income)</i>	_____ Birr	
0304	Does your household own any land (owned or rented) that can be used for agriculture?	1. Yes 2. No _____	Q 0306
0305	How many (local units) of agricultural land does the household own?	1. _____ Units. Write the local unit here (_____) 2. I don't know/Not sure	
0306	How many of the following animals does this household own? Milk cows or oxen? Horses, donkeys, or mules? Goats or sheep? Chickens?	_____ _____ _____ _____	
0307	Does any member of this household own:		
	A kerosene/pressure lamp?	1. Yes 2. No	
	A bed?	1. Yes 2. No	
	A chair?	1. Yes 2. No	
	A table?	1. Yes 2. No	
	A radio?	1. Yes 2. No	
	An electric mitad?	1. Yes 2. No	
	A bicycle?	1. Yes 2. No	
	A mobile telephone?	1. Yes 2. No	
	A non-mobile telephone?	1. Yes 2. No	
	An animal-drawn cart?	1. Yes 2. No	
	A motorcycle?	1. Yes 2. No	
	A television?	1. Yes 2. No	
A boat?	1. Yes 2. No		
A Mill?	1. Yes 2. No		
0308	Does your household have electricity?	1. Yes 2. No	
0309	Does your house have windows?	1. Yes 2. No	
0310	What is the main material of the floor of your house?	1. Natural floor 2. Wood or bamboo floor 3. Finished floor 4. If other specify _____	

0311	What is the main material of the roof your house?	1. Thatched/Leaf 2. Plastic sheet 3. Corrugated iron 4. If other specify _____	
0312	What is the main material of the wall of your house?	1. Bamboo/Wood 2. Stone with mud 3. Trunks with mud 4. Bricks/Cement 5. If other specify _____	
0313	Does any member of this household have an account with a bank/credit association/microfinance?	1. Yes 2. No 3. I don't know/Not sure	
0314	What type of fuel does your household mainly use for cooking?	1. Wood 2. Charcoal 3. Animal dung 4. Kerosene 5. If other specify _____	
4. Reproductive History			
No	Questions	Choices	Remark
0401	When did your last menstrual period start?	1. ___/___/___ (dd/mm/yy) 2. I don't know/Not sure	
0402	How many months pregnant are you now?	1. _____ months 2. I don't remember/Not sure	
0403	Have you ever given birth before?	1. Yes 2. No →	Q 0407
0404	How old were you when you first gave birth?	1. _____ Years 2. I don't remember/Not sure	
0405	When was the last time you gave birth?	1. Before _____ months 2. I don't remember/Not sure	
0406	How many live births did you give before? (Including those who died, if any)	_____ Children/Child	
0407	Have you ever had abortion or stillbirth? (Induced or non induced abortion)	1. Yes 2. No →	Q 0410
0408	If yes how many?	_____ Abortions/Stillbirths	
0409	When was the last time you had abortion or stillbirth?	1. Before _____ months 2. I don't remember/Not sure	
0410	How old were you when you first had your menstruation?	1. _____ Years 2. I don't remember/Not sure	
0411	How old were you when you first got married?	1. _____ Years 2. I don't remember/Not sure	

5. Contraceptive Use Related Information			
No	Questions	Choices	Remark
0501	Have you ever used a modern contraceptive?	1. Yes 2. No _____ →	Q 0601
0502	If yes specify:	1. _____ 2. _____ 3. _____ 4. _____	
0503	Have you ever used a hormonal contraceptive in the last 5 years?	1. Yes 2. No _____ →	Q 0601
0504	If yes, would you please tell me the total duration that you used each of the following hormonal methods?	Combined Oral Pill _____ months	
		Progesterone Only Pill _____ months	
		Injectable Hormone _____ months	
		Norplant _____ months	
0505	How old were you when you started to use the aforementioned hormonal contraceptive methods?	1. _____ Years 2. I didn't use hormonal contraceptives 3. I don't remember/Not sure	
6. Breastfeeding Related Information			
No	Questions	Choices	Remark
0601	Have you ever breastfed?	1. Yes 2. No _____ →	Q 0701
0602	Have you breastfed in the last five years?	1. Yes 2. No _____ →	Q 0701
0603	For how many months did you exclusively breastfeed your recent child?	1. _____ months 2. I don't remember/Not sure	
0604	For a total of how many months did you breastfeed your recent child?	1. _____ months 2. I don't remember/Not sure	
0605	In average how frequently per day did you breastfeed your child?		
	0-6 months	1. _____ times/day 2. Not sure/I don't remember 3. If other specify _____	
	7-11 months	1. _____ times/day 2. Not sure/I don't remember 3. If other specify _____	
	12-23 months	1. _____ times/day 2. Not sure/I don't remember 3. If other specify _____	
0606	How was your feeding pattern during your recent breastfeeding?	1. Less than usual 2. As usual 3. More than usual 4. Not sure/I don't remember	
0607	During your recent breastfeeding, did you get Vitamin A supplementation? (DC: Please show Vitamin A capsule)	1. Yes 2. No 3. Not sure/I don't remember	

7. Information Related to Health Care and Medical Problems During Pregnancy			
No	Questions	Choices	Remark
0701	How long does it take to walk to the nearest health center? <i>(DC: Register the two way walking distance)</i>	1. _____ minutes 2. I don't remember/Not sure	
0702	During this pregnancy, did you have ANC?	1. Yes 2. No _____	Q 0710
0703	If yes, where did you get the ANC service?	1. Health Post 2. Private Clinic 3. Health Center 4. Hospital 5. Community Based providers 6. If other specify _____	
0704	How many months pregnant were you when you first received ANC for this pregnancy?	1. _____ months 2. I don't remember/Not sure	
0705	How many times did you receive ANC so far?	1. _____ times 2. I don't remember/Not sure	
0706	In the ANC you received for the current pregnancy, were any of the following done to you at least once?		
	Height measurement	1. Yes 2. No 3. Not sure/I don't remember	
	Weight measurement	1. Yes 2. No 3. Not sure/I don't remember	
0707	During this pregnancy were you given Fe-Fol Supplementation? <i>(DC: Please show the commonest types of Fe-Fol tablets in the locality)</i>	1. Yes 2. No _____ 3. Not sure/I don't remember _____	Q 0710 Q 0710
0708	If yes, when did you start to take the supplements?	1. Before ____ weeks 2. I don't remember/Not sure	
0709	In the last 28 days (4 weeks) how many Fe-Fol tables did you take?	1. _____ tablets 2. I don't remember/Not sure	
0710	During this pregnancy, did you have vaginal bleeding heavier than a period?	1. Yes 2. No	
0711	During this pregnancy, did you have fever which stayed for more than a day?	1. Yes 2. No _____	Q 0714
0712	If yes, did you seek modern medical care?	1. Yes 2. No _____	Q 0714
0713	If Yes what was the specific diagnosis?	1. _____ 2. Not told/I don't remember/Not sure	
0714	In the preceding one year, have you ever been diagnosed of having malaria?	1. Yes 2. No 3. Not sure/I don't remember	
0715	During this pregnancy, did you have the symptoms of severe headache, generalized body swelling and blurring of vision?	1. Yes 2. No	

0716	During this pregnancy, did you have difficulty with your vision during daylight?	1. Yes 2. No 3. Not sure/I don't remember	
0717	During this pregnancy, did you have difficulty with your vision during dark? <i>(DC: use the local term for night blindness)</i>	1. Yes 2. No 3. Not sure/I don't remember	
0718	In the last one week, did you encounter 3 or more vomiting which stayed for 2 or more days?	1. Yes 2. No	
0719	Do you have any diagnosed medical problem?	1. Yes (Specific) _____ 2. No	
0720	Are you taking any medication now?	1. Yes (Specific) _____ 2. Yes, but I don't know the medication 3. No	
0721	During this pregnancy, did you get any nutrition education/counseling?	1. Yes 2. No _____ →	Q 0801
0722	If yes, who offered you the education/counseling? <i>(DC: Multiple answers possible)</i>	1. Health Extension Workers 2. Community Health Promoters/ Community Nutrition Counselors 3. Health professionals 4. I don't remember/Not sure 5. If other specify _____	

8. Information Related to Dietary Diversity and Dietary Intake

No	Questions	Choices	Remark
0801	What is the most common staple diet in your locality?	1. Enset based foods 2. Cereal based foods 3. If other specify _____	
0802	Which of the following cereals are commonly consumed in your locality? <i>(DC: Multiple answers possible)</i>	1. Maize 2. Sorghum 3. Teff 4. Wheat 5. Barely 6. Finger millet 7. If other specify _____	
0803	Which of the following legumes are commonly consumed in your locality? <i>(DC: Multiple answers possible)</i>	1. Broad bean 2. Peas 3. Chickpea 4. Haricot bean 5. Kidney bean	
0804	What do you commonly grow on your farm land?	1. _____ 2. _____ 3. _____ 4. _____	
0805	How do you rate your dietary intake during this pregnancy? <i>(DC: Read out the choices)</i>	1. Less than usual 2. As usual 3. More than usual 4. Not sure/I don't remember	

0806	Are you currently receiving food aid from a government or NGO source?	1. Yes 2. No →	Q 0809
0807	If yes, when did you last receive this relief food?	1. Before ____ weeks 2. I don't remember/Not sure	
0808	What type of food aid did you receive? <i>(DC: Multiple answers possible)</i>	1. Wheat 2. Maize 3. Legumes 4. Oil 5. Blended Food 6. If other specify _____	
0809	Do you have backyard garden to grow fruits and vegetables?	1. Yes 2. No →	Q 0811
0810	If yes what type of fruit/vegetable is grown now?	1. _____ 2. _____ 3. _____ 4. _____	
0811	I would like to ask you about the types of foods that you ate over the past 24 hours, from sunrise yesterday to sunrise today. Did you take any of the following foods?		
	Any food made from grains (Millet, Sorghum, Maize, Rice, Wheat, Teff etc) including Enjera, Bread and Biscuits?	1. Yes 2. No	
	Any food made from legumes like Beans, Peas, Lentils, or Nuts?	1. Yes 2. No	
	Any food made from roots or tubers? (Potatoes, Cassava, Enset, or other local roots or tubers)?	1. Yes 2. No	
	Any food made from Pumpkins, Carrots, red sweet Potatoes, green leafy vegetables (such as Kale and Pepper), Mango, and Papaya?	1. Yes 2. No	
	Any other fruit?	1. Yes 2. No	
	Any other vegetable?	1. Yes 2. No	
	Any meat (Beef, Pork, Lamb etc)?	1. Yes 2. No	
	Any Cheese, Yogurt, Milk or other milk products?	1. Yes 2. No	
	Any Eggs?	1. Yes 2. No	
	Any fresh or dried Fish?	1. Yes 2. No	
	Any food made with Oil, Fat or Butter?	1. Yes 2. No	
	Any Sugar or Honey?	1. Yes 2. No	
	Any other foods?	1. Yes 2. No	

0812	How many meals and snacks did you eat between yesterday's and today's sunrise?	1. _____ times 2. I don't remember/Not sure	
0813	On average, how many cups of tea do you take in a typical day? (DC: Estimate in standard 70 ml coffee cup)	1. _____ Cups 2. I don't remember/Not sure	
0814	On average, how many cups of coffee do you take in a typical day? (DC: Estimate in standard 70 ml coffee cup)	1. _____ Cups 2. I don't remember/Not sure	
0815	On average, how many cups of alcohol (mention some local alcohol drinks) do you take in a typical day? (DC: Estimate in standard 70 ml coffee cup)	1. _____ Cups 2. I don't remember/Not sure	
0816	DC: take a teaspoon of table salt from the household the pregnant women belong and estimate the level of iodization.	1. No iodine 2. Less than 15 PPM 3. Greater than 15 PPM	
0817	In your locality, is there any food which is prohibited during pregnancy due to cultural or religious beliefs?	1. Yes 2. No → 3. Not sure/I don't know →	Q 0821 Q 0821
0818	If yes, specify them.	1. _____ 2. _____ 3. _____	
0819	During this pregnancy were you prohibited (or restricted yourself) from taking one or more food items due to cultural or religious beliefs?	1. Yes 2. No →	Q 0821
0820	If yes, specify them.	1. _____ 2. _____ 3. _____	
0821	Now I want to ask you how many times you consumed the following food items in the preceding one week starting from _____ morning to the sunrise today.		
	Food prepared from any meat?	1. _____ times	2. I don't remember/Not sure
	Food prepared from any milk or milk products?	1. _____ times	2. I don't remember/Not sure
	Food prepared from egg?	1. _____ times	2. I don't remember/Not sure
	Food prepared from fish?	1. _____ times	2. I don't remember/Not sure
	Enset based foods (like Kocho, Kuncihsa, Bulla etc...)	1. _____ times	2. I don't remember/Not sure
	Any food made from roots or tubers? (Potatoes, Cassava, etc excluding Enset)?	1. _____ times	2. I don't remember/Not sure
	Any food made from grains (Millet, Sorghum, Maize, Rice, Wheat, Teff etc) including Enjera, Bread and Biscuits?	1. _____ times	2. I don't remember/Not sure
	Any food made from legumes?	1. _____ times	2. I don't remember/Not sure
	Any food made from Pumpkins, Carrots, red sweet Potatoes, green leafy vegetables (such as Kale and Pepper), Mango, and Papaya?	1. _____ times	2. I don't remember/Not sure
	Any other fruit?	1. _____ times	2. I don't remember/Not sure
	Any other vegetable?	1. _____ times	2. I don't remember/Not sure
	Any food made with Oil, Fat or Butter?	1. _____ times	2. I don't remember/Not sure

0822	While preparing food for your household do you commonly perform the following food processing procedures?	
	Removal of the bran of cereals and legumes through pounding, manual milling, etc.	1. Yes 2. No
	Fermentation of cereals, legumes for days.	1. Yes 2. No
	Fermentation of Enset for days.	1. Yes 2. No
	Soaking cereals and legumes for hours.	1. Yes 2. No
	Germination of cereals and legumes.	1. Yes 2. No

9. Information Related to Maternal Workload

No	Questions	Choices	Remark
0902	In the last one week, how many times did you participate in any of the following activities?		
	Fetching water (walking distance > 30 mins)?	1. _____ times 2. I don't remember/Not sure	
	Collecting firewood/Cow dung (walking distance > 30 mins)?	1. _____ times 2. I don't remember/Not sure	
	Washing clothes?	1. _____ times 2. I don't remember/Not sure	
	Land preparation/leveling?	1. _____ times 2. I don't remember/Not sure	
	Plowing and digging?	1. _____ times 2. I don't remember/Not sure	
	Weeding?	1. _____ times 2. I don't remember/Not sure	
	Manual mowing?	1. _____ times 2. I don't remember/Not sure	
	Threshing?	1. _____ times 2. I don't remember/Not sure	
	Grinding grain?	1. _____ times 2. I don't remember/Not sure	
	Pounding grain?	1. _____ times 2. I don't remember/Not sure	
	Preparing Enset?	1. _____ times 2. I don't remember/Not sure	
	Terracing?	1. _____ times 2. I don't remember/Not sure	
0902	Compared to the pre-pregnancy situation how do you rate your work burden during this pregnancy? (DC: Read out the choices)	1. As usual 2. More than usual 3. Less than usual	

10. Information Related to Household Food Security			
No	Questions	Choices	Remark
1001	In the past four weeks, did you worry that your household would not have enough food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1002	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?	1. Yes 2. No 3. Not Sure/Don't Remember	
1003	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?	1. Yes 2. No 3. Not Sure/Don't Remember	
1004	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1005	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1006	In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1007	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1008	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?	1. Yes 2. No 3. Not Sure/Don't Remember	
1009	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?	1. Yes 2. No 3. Not Sure/Don't Remember	

Thank you for your cooperation

Checklist format to be filled by the data collector after physical examination of the mother

1. Body Height _____ cms
2. Body Weight _____ kgs
3. Middle Upper Arm Circumference _____ cms
4. Fundal Height _____ weeks
5. Goiter Present _____ Absent _____
6. If present the stage _____ stage
7. Pallor Present _____ Absent _____
8. Bitot's spot Present _____ Absent _____

Checklist format to be filled by the lab technician after lab analysis

1. Hemoglobin level _____ gm/l
2. Serum zinc level _____ mg/L
3. Serum retinol level _____ μ mol/L

Annex 4: Amharic Checklist

የመጠይቁ መለያ ቁጥር: _____

ከወልደት በኋላ ስለ እንጉረዳና ልጅ ሁኔታ የመመዘገቢያ ቅጽ

የጤና ኤክስፐርት ስራ-ተኛው ስም: _____
 የተመደበው የበጎ ፍቃድ ጤና መልክተኛ ስም: _____
 ወረዳ: _____ ቀበሌ: _____

I. መሰረታዊ መረጃና አድራሻ፤

1. የናቲቱ ስም: _____
2. እድሜ: _____
3. ትወልዳለች ተብሎ የሚጠበቅበት ጊዜ: _____
4. አድራሻ: (በአካባቢው በቀላሉ የሚታወስ/በሚታወቅ የቦታ መለያ ይመዘገቡ):

II. የሚከተሉትን መረጃዎች እናት እንደወለደች በሶስት ቀን ውስጥ ይመዘገቡ፤

5. ልጅ የተወለደበት ቀንና ሰዓት: _____ ቀን: _____ ሰዓት: _____
6. ልጅ የተጎበኘበት (የተለካበት) ቀንና ሰዓት: _____ ቀን: _____ ሰዓት: _____
7. የልጅ ክብደት በኪ.ግ. (#.##): _____
8. የልጅ ቁመት በሴ.ሜ. (###): _____
9. የልጅ የራስ ዙር ልክ በሴ.ሜ. (##.#): _____
10. የልጅ ያታ:

ሀ. ወንድ	ለ. ሴት
--------	-------

III. ልጁ ሲወለድ ስለነበረው ምጥ የሚከተለውን መረጃ ይመዘገቡ፤

11. ምጡ መች ጀመረ? _____ ቀን: _____ ሰዓት: _____
12. የሽርጥ ወሃ መች ፈሰሰ? _____ ቀን: _____ ሰዓት: _____
13. በእርግዝና፣ ምጥ ወይም ወልደት ጊዜ የነበረ ማንኛውም የጤና ችግር ከነበረ ይመዘገቡ:

14. የእርግዝናዎ አይነት፤

- | | |
|-------------|--------|
| ሀ. መንታ ያልሆነ | ለ. መንታ |
|-------------|--------|

IV. ስለተወለደው ልጅ የሚከተሉትን መረጃዎች ይመዘገቡ፤

15. ልጁ/ልጅቷ መች ጡት መጥባት ጀመረ/ች?
 ሀ. እንደተወለደ/ች በእንድ ሰዓት ውስጥ
 ለ. እንደተወለደ/ች ከእንድ እስከ ሃያ አራት ሰዓት ባለው ጊዜ ውስጥ
 ሐ. ከሃያ አራት ሰዓት በኋላ
16. ልጁ/ልጅቷ ከተወለደ/ች በኋላ ከጡት ወተት ወጭ የተመገበው/የጠጣው/ችው ምግብ ነበር?
 ሀ. አዎን (ይጠቀስ: _____) ለ. የለም
17. ልጁ/ልጅቷ ከተወለደ/ች ጊዜ ጀምሮ ስንት ጊዜ ጡት ጠባ/ች? _____ ጊዜ
18. ልጁ/ልጅቷ ከተወለደ/ች በኋላ ከታች ከተዘረዘሩት ውስጥ ያጋጠመ ችግር ነበር?

ማስመለስ?	ሀ. አዎን	ለ. የለም
ማስቀመጥ?	ሀ. አዎን	ለ. የለም
ለመጥባት መቸገር?	ሀ. አዎን	ለ. የለም
ትኩሳት?	ሀ. አዎን	ለ. የለም

Annex 5: English Checklist

Questionnaire identification number: _____

Checklist for registering the health of the mother and baby after birth

Name of the health extension worker: _____

Name of the assigned CHP: _____

Woreda: _____ Kebele: _____

I. Identification information:

1. Name of the mother: _____
2. Age: _____
3. Expected date of delivery: _____
4. Address: (register local land mark to identify the usual address of the mother):

II. Fill the following information within the first three days of birth.

5. Exact time of birth: Date: _____ Time: _____
6. Exact time of measurement: Date: _____ Time: _____
7. Birth weight of the baby in kgs (###): _____
8. Length of the baby in cms (###): _____
9. Head circumference of the baby in cms (###): _____
10. Sex of the baby: (a) Male (b) Female

III. Fill the following information about the labor and birth.

11. When did the labor start? Date: _____ Time: _____
12. When did the amniotic membrane rupture? Date: _____ Time: _____
13. If there was any pregnancy, labor or delivery complication describe it here:

14. Type of labor:
(a) Singleton births (b) Twin birth (c) If other specify: _____

IV. Fill the following information about the post natal situation of the newborn.

15. When did the newborn start breastfeeding?
(a) Immediately after birth (within an hour of birth)
(b) 1-24 hrs after birth
(c) After 24 hours
16. Did anything was given to the newborn other than breast milk?
(a) Yes (specify: _____) (b) No
17. How many times did the mother breastfed her newborn so far? _____ times.
18. Did the newborn have any of the following problems so far?
 - a. Vomiting? 1. Yes 2. No
 - b. Diarrhea? 1. Yes 2. No
 - c. Difficulty in breathing? 1. Yes 2. No
 - d. Fever? 1. Yes 2. No

Letter of Declaration

I, the undersigned, declared that this is my original work, has never been presented in this or any other University, and that all the resources and materials used for the thesis, have been fully acknowledged.

Name: Samson Gebremedhin Gebrelassie

Signature: _____

Date: _____

Place: Addis Ababa

Date of submission: _____

This dissertation has been submitted for examination with my approval as PhD Supervisor.

Name: Dr. Fikre Engusellassie Gashe

Signature: _____

Date: _____