Addis Ababa University

College of Natural Science

Center for Food Science and Nutrition

Prevalence and associated risk factors of zinc deficiency among pregnant women in Selected Health Centers in Addis Ababa, Ethiopia

By: Kebebew Regassa

Advisor: Dr Dawd Gashu

A thesis Submitted to the School of Graduate Studies, Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Nutrition.

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Declaration

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Table of Contents

Acronyms and Abbreviations

AAS	Atomic Absorption Spectrometry
AAU	Addis Ababa University
AI	Adequate Intake
ANC	Antenatal Care
ANOVA	Analysis of Variance
CDC	Center for Disease Prevention and Control
CI	Confidence Interval
DD	Dietary Diversity
DHS	Demography and Health Survey
EDHS	Ethiopia Demographic and Health Survey
EPHI	Ethiopia Public Health Institute
FANTA	Food and Nutrition Technical Assistance
FFQ	Food Frequency Questionnaire
FGD	Frequency Group Distribution
FNB	Food and Nutrition Board
IDDS	Individual Dietary Diversity Score
IGF-1	Insulin likes Growth Factor
IRB	Institutional Review Board
IZiNCG	International Zinc and Nutrition Consultative Group
MOH	Ministry of Health
NGO	Non-Governmental Organization
PI	Principal Investigator
PPM	Parts Per Million
RBP	Retinol Binding Protein
RCTs	Randomized Control Trials

RDA	Recommended Dietary Allowances
RPM	Revolution per Minute
SD	Standard Deviation
SPSS	Statistical Package for Social Science
UNICEF	United Nation Children's Fund
USNHANES	United States National Health and Nutrition Examination Survey
WHO	World Health Organization

List of Table

List of figures

Abstract

Background: Many studies reveal that zinc deficiency during pregnancy leads to diverse pregnancy complications. However, evidences on the associated risk factors of zinc deficiency among pregnant women are limited and not conclusive. The aim of the present study was to assess the prevalence and associated risk factors of zinc deficiency among pregnant women in Addis Ababa, Ethiopia.

Methods: Randomly selected 403 women were included in cross-sectional study conducted in Addis Ababa in July-August, 2016. Data on associated risk factors of zinc deficiency were gathered using a structured questionnaire. Blood samples were collected to analyze biochemical indicators. Serum zinc concentration was measured using Atomic Absorption Spectrometry. Statistical analysis was done using logistic regression methods. P-value<0.05at 95% confidence interval was considered statistically.

Results: The mean serum zinc concentration was 63.68(±27.3) µg/dl (95% CI: 21.0 to 194.7µg/dl).The prevalence of zinc deficiency among pregnant women was 39.5% (95% CI: 34.5% – 44.2%).Morbidity during pregnancy, low intakes of foods of animal origin, coffee intake and elevated C-reactive protein were pertinent risk factor significantly associated with zinc deficiency. The risk of zinc deficiency of pregnant women with elevated CRP was two and half times higher as compared to pregnant women with normal CRP [AOR=2.48;95% (1.45, 4.24)].The risk of zinc deficiency was two times [AOR = 1.98; 95% CI (1.25, 3.14)] higher than

pregnant women who had morbidity. The risk of zinc deficiency for pregnant women who didn't consume animal source foods were [AOR = 2.11; 95% CI (1.30, 3.42)] two times higher, as compared to pregnant women who consumed animal source foods. Compared to pregnant women who didn't not consume coffee, the risk of zinc deficiency was 2.12 times higher among those who consumed coffee [AOR = 2.12; 95 % CI (1.39, 3.42)]. Zinc level was positively associated with hemoglobin concentrations and consumption of animal source foods. Coffee intake, morbidity, and elevated CRP were negatively associated with zinc concentration.

Conclusion: zinc deficiency is of public concern in the area. The problems could be combated through increasing nutrition knowledge and practices concerning consumption of zinc rich and bioavailable foods, optimal diversified foods, and use of home based phytate reduction methods, agricultural biofortification based approaches and livelihood promotion should be considered

1. Introduction

1.1. Background

Zinc is an essential trace element with diverse and metabolic functions [1]. It participates in major biochemical pathways and plays several roles such as cell division, physical growth, immunity, reproductive function, neuron function and behavioral development [2, 3]. In addition, the element is important for normal blood sugar balance, proper appetite, sense of taste and smell, and proper function of the sense of vision [2-4].

Moreover, more than 300 different enzymes have zinc as a cofactor for their catalytic purpose. It is approximated that about 10% of the chemical reactions in the body are catalyzed by enzymes that require the presence of zinc. Hydrolases are the most common zinc enzymes in all domains of life [2, 4].

Zinc deficiency is a wide spread public health problem in the world [5, 6]. In spite of the proven benefits of adequate zinc nutrition, approximately 2billion people in the world still remain at risk of zinc deficiency [6, 7]. The three major health problems associated to chronic zinc deficiency which are most common in developing countries are growth retardation, susceptibility to infections and cognitive impairment [8].

The limited available reports on the prevalence of zinc deficiency in pregnant women from Ethiopia suggest that the deficiency is a public health concern. In Ethiopia, few studies determined the prevalence of zinc deficiency in pregnant women and reported the presence of 53% to 76% zinc deficiency [9].

The effect of zinc on maternal health and pregnancy out comes have been studied in multiple observational and interventional studies [10]. Zinc deficiency in pregnant mothers has been associated with a wide range of complications including low birth weight, labor and delivery complications, congenital anomalies, hypertension, placental abruption, premature delivery, increased neotal morbidity, intrauterine growth retardation, and poor neurobehavioral development [2, 11].

Few studies attempted to identify associated risk factors related to prenatal zinc deficiency in Ethiopia. The commonly indicated risk factors are dietary [1, 9, 21], morbidity [9], demography [1, 9], and socio-economic status [1]. However, as studies are few, they are not conclusive. In addition, available studies concentrate on zinc status of women and children from rural areas. Therefore, this study was designed to assess the prevalence and associated risk factors of zinc deficiency in pregnant women from Addis Ababa.

1.2. Problem Statement

Zinc is an essential mineral that play structural, regulatory, catalytic role in the body. In addition, it is critical for normal immune functions [3]. World Health organization (WHO) approximated that zinc deficiency affects 31% of the population globally ranging from 4%-73% in different regions of the world. In developing countries zinc deficiency is a significant risk factor for the global burden of disease [12].The major consequences of zinc deficiency are prenatal complications, increase risk of morbidity and mortality by impairing immunity, neuron-sensory changes, delayed wound healing, growth retardation, male hypogonadism, impaired reproduction, and cognition [8,10,13].

According to global and regional child mortality and burden of disease report, Ethiopia is included in the five countries that make 47% of the child deaths due to zinc deficiency [14]. According to the Ethiopia Demographic and Health Survey (EDHS) 2011 report, about 44% of the under five children are stunted which is a proxy indicator of the magnitude of zinc deficiency in the country [15]

Pregnant women are facing zinc deficiency more than the other groups due to physiological changes that require an increased zinc to accommodate normal zinc demand. Thus on circumstances where pregnant women do not get this additional requirement deficiency could happen that complicate pregnancy out comes and delivery [8, 16]. Studies that determine zinc status in pregnant women in Ethiopia concentrate in rural area which may not also represent the situation in women from urban areas. This is because women from urban areas are socially and economically advantageous thus could have better access to zinc rich diets. In addition, they have better access to health services and communication infrastructures to get knowledge hence are expected to be less vulnerable to etiological factors for zinc deficiency. Therefore, this study designed to assess prevalence and associated risk factors of zinc deficiency during pregnancy [1, 9].

1.3. Significance of the Study

The study generates information on magnitude of zinc status and how dietary, morbidity, socio-economic, and demographic factors influencing serum zinc levels among pregnant women. This may be useful in formulating strategic polices to address zinc deficiency and maternal child nutrition and health care education. The finding from study may contribute to knowledge on maternal and child healthcare. The knowledge may be useful to guide the design nutrition program in the Ministry of health (MOH), and any organization that intends to design interventions program, in Ethiopia and worldwide.

1.4. Objective of Study

1.4.1. General Objective

To assess prevalence and associated factors of zinc deficiency among pregnant women in Selected Health Centers in Addis Ababa, Ethiopia

1.4.2. Specific Objectives

- To determine the prevalence of zinc deficiency among pregnant women
- To identify factors associated with zinc deficiency among pregnant women
- To evaluate the relationship between hemoglobin and zinc status

2. Literature Review

2.1. Biological Significance of Zinc

Zinc is an essential element involved in multiple aspect of cellular metabolism which attained prominence in human health [10].Enzymes in the body requires zinc more than all trace minerals combined. Zinc is also important to catalyze chemical reactions, cell membranes synthesis and normal function of proteins in the body. Furthermore, it is important to read DNA instructions, cell signaling, hormone release, and nerve impulse transmissions [4, 17and 18].It is also important to maintain skin and mucosal membranes which are important for wound healing. It is also important for protection against respiratory tract infections and replication of the rhinovirus [3, 18].Its availability affects cell-signaling systems that coordinate the response to the growth-regulating hormone -Insulin like Growth Factor (IGF-1).Without zinc; this growth cannot do its job leading to stunted growth in young children. Bone growth is also affected due to zinc dependent enzymes and hormones required for development [2, 18, and 19]. The mineral is also crucial for normal functioning of the prostate gland to manufacture testosterone and production of healthy sperm [2, 3].

Zinc is also required by normal functioning of retinol binding protein (RBP) and the deficiency can cause a secondary vitamin A deficiency despite of vitamin A adequate nutrition. It is also important to change retinol to retinal which is require to create rhodopsin, the protein in the eye that absorb light and so, a lack of zinc is potential to cause night blindness. Low zinc status can cause poor thymic development and a poorly developed thymus cause weak T-cells that are unable recognize and fight of infection [2, 18]. Zinc can neutralize free radicals and may reduce or even help prevent some of the damage they cause during the pregnancy (19).

2.2. Zinc Metabolism

Zinc is absorbed in the small intestine and binds to the storage protein metallothionen. If the body does need zinc, metallothionen hands zinc to albumin and transferrin for transport to tissue that need it mostly muscle and bone tissues. Absorption varies depending on zinc content and diet composition. Soluble organic substances like amino acids 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 absorption. If the body does not need zinc, it is sloughed off along with dead cells and excreted by the pancreases in to the intestinal tract and then leaves the body through feces and to the lesser extent in urine and sweat .The level of excretion is affected by dietary intake. Starvation and muscle catabolism increase zinc loss in urine. Strenuous exercise and elevated ambient temperatures lead to losses through perspiration. In men loss of zinc in semen can also be substantial [3, 10].

2.3. Zinc Deficiency

The use of zinc for human health was first recognized in 1963 when zinc deficiency was identified as a major etiological factor in the syndrome of "Adolescent nutritional dwarfism" in Egypt and Iran. Later, the deficiency of zinc was identified among patients' of Acrodermatitisenteropathica and people that exclusively rely on intravenous feeding. In 1970sand 80s, randomized control trials (RCTs) confirmed that zinc deficiency is a causative to growth retardation in children. Studies carried out throughout1990s and beyond contributed to the documentation of the etiological role of zinc in many clinical outcomes including neuropsychological functions and immune impairment [10, 16].

Zinc deficiency during adolescence can lead to delayed puberty [2, 3].A decrease in zinc nutrition could cause a decrease in insulin response which in turn destabilizes blood sugar levels. Decrease in adequate zinc levels create a decrease in metabolic rate. Low levels of zinc are also associated with impaired thyroid metabolism despite of adequate iodine nutrition. This is because zinc is essential in assistance of iodine to thyroid to secrete thyroid hormone [5].Lack of zinc also causes poor retina and macula functioning and its level in the retina decreases with age also impacting vision [2].Symptoms of zinc deficiency is poor appetite, impair of taste and smell, slow wound healing, hair loss, impaired cell-mediated immunity, depression, diarrhea, lethargy [2, 3, 8].

Children and mothers are commonly considered as vulnerable groups for zinc deficiency. The process of developing zinc storage happen during the third trimester of pregnancy leaving preterm infants in particular at risk of zinc deficiency [2,3].In addition, on the reason that breast milk can no longer provide adequate zinc beyond six months and commonly complementary foods is zinc deficient, hence infants older than six months are affected by zinc deficiency. In pregnant women the newly synthesized fetal and maternal tissue during pregnancy imposes additional physiological requirements for zinc since the fetus require high levels of zinc [2, 3, and 20].

2.4. Prevalence of Zinc Deficiency during pregnancy

According to WHO, one third of the world population is at risk of zinc deficiency and the risk ranges from 4-73% across various regions. The prevalence is low (4-7%) in North America and Europe, and high in North Africa and Eastern Mediterranean (25-52%), South and Central America (68%), Sub-Saharan Africa (37-62%), and South and Southeast Asia (34-73%) [21].

A study carried out among women in Nepal reported a 75% zinc deficiency prevalent among women of reproductive age. This was related to adverse poor pregnancy out comes and prevalent risk of infections [22].Several studies illustrated extremely high prevalence of zinc deficiency 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 [21].

Studies in India, Peru, Iran, Indonesia, Vietnam, and Bangladesh reported 65%(23), 60%(16), 49%(24), 22%(25), 29%(26), and 14.7%(27),respectively, zinc deficiency prevalence among pregnant mothers. Substantial burden has also been reported in developed countries like Sweden (18%) (28).

There are limited reports on national prevalence of zinc deficiency in Africa rather the only existing country specific information is the estimate from IZiNCG which is not based on serum data [10].According to the estimate, all people of Africa countries are either in high or intermediate risk category for zinc deficiency. Ethiopia was also classified into the intermediate risk group [10]. Pocket studies from Nigeria45.8%(29),Malawi36%(30), Cameroon82%(31),Eastern Sudan38%(32), and Kenya66.9%(20) reported zinc deficiency in pregnant women.

The limited available data suggest that zinc deficiency is of public health concern in Ethiopian pregnant mothers [10, 33]. In Ethiopia, few studies determined the prevalence of zinc deficiency in pregnant women and obtained results ranging from 53to76% [9].All studies consistently indicated that zinc deficiency is public health significance in the country.

2.5. Associated Risk Factors of Zinc Deficiency during Pregnancy

The etiology of zinc deficiency may be dietary, physiologic, and also health related factors. Dietary deficiency can be due to low zinc in foods consumed or low bioavailability due to the presence of anti-nutrient interfering zinc absorption or combinations of these dietary factors [34, 45].Low intakes of dietary zinc are commonly associated with low energy intakes that caused by food insecurity in many low income countries. Inadequate intake of animal source foods, caused by economic or religious problems, may also contribute to low zinc intakes. This is because meat, poultry, and fish are rich sources of readily absorbable zinc. In some geographical areas, low soil zinc concentrations may cause lower zinc content of staple foods [3,20].In many low income countries, most of their staples diets are derived from plant-based foods thus are prone to zinc deficiency. Plant-based foods contain high amount of phytic acid which is the major storage form of phosphorus in legumes that chelate minerals such as zinc. It is the major storage form of phosphorus in legumes and cereals is the only considerable dietary factor that inhibits zinc absorption, especially when foods are low animal food sources [20, 45].

Amounts of phytate and zinc and their molar proportion in the foods is proxy indicate of zinc bioavailability in the diets. Diets with phytate/zinc molar ratio greater than 15 are low bioavailability with zinc absorption 15%.Diets like a cereal based with low intake of animal sources is found to be in this range .Diets with phytate/zinc molar ratio range between 5-15 are a medium bioavailable with 30%of zinc bioavailability. Diets that classified into this category are mixed diets and vegetarian whose diets includes milk or milk products and eggs .Diets with molar ratio <5are of high zinc bioavailability with 50% zinc absorption ,which includes diets derived from animal sources and diets with low quantity of fiber[20].

Content of phytate can be decreased using food processing methods such as soaking, fermentation and germination:

- Soaking is a method to decrease the phytate content of certain cereals and legumes. About 10-70% of phytate in these staples is stored in water soluble form and hence can be removed by diffusion.
- Fermentation induces phytate hydrolysis through the action of microbial phytase which can be come either from the micro flora on the surface of the seeds or by introducing with microbial starter.
- Germination of seeds is an acting of seeds to germinate for 2-3 days that can enhance the breaking of phytate by 23-88%, which are affected by the type of grain [2, 10].

Several non-nutritional importance may also be contributed by community –based dietary mechanisms such as empowerment of women ,training ,and income generation .To be seen successful this strategy ;It need major changes in attitudes and food–related behaviors and practices that needed for effective behavior change and communication. In addition, it must be work with ongoing national agriculture, food, nutrition and health education programs and implemented using participatory approach to ensure their acceptability, adoption, and sustainability [2, 10, and34].

Pregnancy results in an elevation of plasma volume and lowered levels of circulating-binding proteins thus is a risk factor for zinc deficiency suggesting additional zinc nutrition during this time is necessary. If pregnant mother do not get this increased requirement for zinc during pregnancy the body cannot accommodate normal zinc demand and its deficiency could affect pregnancy out comes and delivery. In many low-income countries, high physiologic changes can be aggravated by under nutrition, resulting into micronutrients deficiency like zinc. In pregnant women, moderate to severe deficiencies of zinc have been demonstrated to increase risk of low birth weight, pregnancy complications and birth defects [16, 33].

Study conducted in Pakistan in 2011showed that from 791 pregnant women, 48.3 %(< 60µg/dl) were zinc deficient. This was due to mainly dietary staples of the Pakistan populations are poor zinc content [22]. Study conducted to assess zinc deficiency among pregnant women in Nigeria show that 45.8%ofpregnant women were zinc deficient attributed mainly to low frequency of meat, dairy products, nuts and vegetables consumption [29].In addition, zinc deficiency among pregnant women in Kenya was reported to be 66.9%. Parity was negatively associated with serum zinc levels among the study population ($p<0.05$)[20].

In Ethiopia, information on the prevalence of zinc deficiency is limited. A study conducted in Sidama showed that 72% of pregnant women were zinc deficient and the major cause for this was due low or absence of the inclusion of cellular protein in the diet. On the other, their diet was characterized by cereals and starchy tubers such as Enset (false banana) which inherently contain high level of zinc absorption inhibitors [35]. In addition, a study conducted at rural Sidama to determine the prevalence of prenatal zinc deficiency and its associations with socio-demography, dietary and health care indicated the presence of 53% prevalence of zinc deficiency among pregnant women. The major cause were dietary factors like household food insecurity, low dietary diversity and consumption of inadequate animal source foods, low maternal education, age and parity [1].Furthermore, a study conducted in Gondar to determine the prevalence of prenatal zinc deficiency and its associations with socio-demography, dietary and health care were indicated 57.4% prevalence of zinc deficiency pregnant women. The major causes were too close birth intervals, low intake of animal foods, inadequate dietary diversity, and lack nutritional education [9].

Serum zinc concentration is independently affected by acute inflammation/infection, diurnal variation and fasting. The concentration is decrease during acute infection/inflammation probably due to the redistribution of zinc from plasma to the tissue. The concentration also indicates diurnal variation principally because of the effect of food ingestion. Following a meal, there is immediate initial increase serum zinc followed by a gradual decrease. During an overnight fasting, the concentration steadily increases to reach the highest level in the morning [10].

2.6. Zinc Food Sources

Red meat, whole-grain cereals, legumes, pulse and milk give the highest concentrations of zinc(25-50 mg/kg).Processed cereals, polished rice, and meat with the high fat content have moderate content (10-25 mg/kg).Fish, roots, tubers, green leafy vegetables and fruits are only modest sources(<10mg/kg).Separated fats and oils and sugar have very low zinc content. The extent of bioavailability of zinc in plant source diets is limited due to the presence of high phytate that inhibit zinc absorption [36].

The bioavailability of zinc is higher in animal products due to the absence of compounds that inhibit zinc absorption (i.e. phytates and oxalates) and the presence of certain amino acids (i.e. cysteine and methionine) that improve zinc absorption. Good zinc sources among animal source foods include: goat, beef, shellfish, liver, eggs and good plants sources include whole grains, peanut. Breast milk is good sources of zinc for infant. It is not affected by maternal zinc status much[2].The enzymatic action of yeast decreases the level of phytic acid in foods, therefore, leavened whole-grain breads have more bioavailable zinc than unleavened whole grain breads [2,36,37].

2.7. Strategies to Enhance zinc Status

Potential strategies to address zinc nutrition include food diversification, supplementation, and fortification and the choice of the specific strategies depending on the magnitude of risk, life stage group, and available setting [2, 13].

2.7.1. Dietary diversification

Dietary diversification includes changes in food production and food selection patterns as well as traditional household methods for preparing and processing indigenous foods. The ultimate goal is to improve the availability, access, and utilization of foods with a high concentration and bioavailability of micronutrients all the time. Dietary diversification used to increase both zinc-rich foods and zinc absorption enhancers at the same time deceasing phytate, the potent inhibitor of zinc absorption [2, 13].There are a different ways used in dietary diversification/modification strategy. They include :(a)increasing the energy density of diets;(b)increasing the production and consumption of foods with a high content of micronutrients including zinc;(c)increasing the production and consumption of foods known to enhance zinc bioavailability [2].

2.7.2. Supplementation

Zinc is supplemented in the form of capsule or tablets to targeted groups. Supplementation is useful to targeting vulnerable groups, which are at high risk of micronutrient deficiencies [3].It is restricted to those targeted groups because it does not address the root cause of the deficiency.

However, it offers a relatively short term solutions for micronutrient deficiencies [13, 38].Supplementation only works if the supplements are available and accessible and intended individual take them [13].

Strong evidences exists that zinc supplementation improve diarrheal episode and enhances physical growth in stunted and low birth weight babies. However its significance in preventing pregnancy complication is equivocal. The major challenges of zinc supplementation method are uncertainty in effectiveness[10], for examples, infants born to zinc supplemented women had greater weight gain, higher calf, and chest circumference, and more calf muscle area than children born to women without zinc supplementation [39]and zinc supplementation during pregnancy is associated with significant reduction in preterm births without an effect on infant birth weight[40,41],bioavailability, side effect and compliance; donor dependency, and added cost and burden on the health care system [10].

2.7.3. Fortification:

Fortification is addition of micronutrients to processed foods at household and industrial level [38]. Zinc fortification is a cost effective and sustainable method that reverses the root cause of the problems for improving zinc status in countries where zinc deficiency is prevalent. It does not need to alter in the existing food beliefs and does not impose a burden on the health aspects. Flour, bakery goods, breakfast cereals, cereals, and infant formulas are commonly fortified with zinc. Fortification of cereals in Ghana and Mexico has also indicated encouraging out comes. Major problems of this methods are lack of industrially prepared and universally eaten foods, technological and economic feasibility, lack of bioavailability and difficulty in balancing optimal and excessive extent [2,10]. In developing countries zinc fortification is restricted to solving problems related to nutritional need of population in refugee camps and pregnant mothers and children enrolled in supplementary feeding programs [10].Fortification methods could be applied at the national level with the objective of increasing the zinc status of the population, or targeted group which are at risk [2].Therefore, fortification in of staple foods require active governmental leadership, policy, and political will [3,42].

In the long-term, biofortification of staple crops improve both micronutrient status and bioavailability. Bio fortification does not depend on a food vehicle being centrally processed.

Biofortification implemented in three ways:

a) Agronomic practices is application of soil fertilizers to enhance zinc content in staple crops when staple crops are grown in low zinc content soil.

b) Conventional plant breeding is harvesting species that used to enhance zinc concentrations in seeds or used to produce new varieties with reduced phytic acid content to improve zinc absorption.

c) Genetic modifications involving gene insertion or induced mutations [2, 3].

Dietary diversification/modification and bio fortification are commonly used for rural poor who consume staple foods from local or self –production that coverage left by fortification due to accessibility, affordability, and reliance on continuing donor support [3].

2.8. Zinc Dietary Requirements

High percentage, 82% of women worldwide have inadequate dietary zinc intake. The recommended dietary allowance (RDA) of zinc differs by age, gender, pregnancy and breastfeeding status. In both sexes the requirement is higher during puberty. Pregnant and non-lactating women require 8mg/day. Pregnant women and lactating women instead require 3 and 4 mg more zinc daily, respectively [36].The RDA for zinc of a population is indicated in the table 1 and estimated physiological requirements for absorbed zinc by age group and sex are indicated in the table 2, respectively.

Table1. The RDA for Zinc (sources:http://www.dietbites.com/Vitamins-Minerals-Facts/Zinc-1.html,2010)

Life Stage	**Age**	**Males (mg/day)**	**Females (mg/day)**
Infants	0-6months	2(AI)	2(AI)
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger	-	12
Pregnancy	19 years and older	-	11
Breast-feeding	18 years and younger	-	13
Breast-feeding	19 years and older	-	12

Table 2: Estimated physiological requirements for absorbed zinc by age group and sex (Roohani., et al.2013[3])

Variables Age	WHO		Variables Age	FNB		IziNCG	
	Reference Wt. (kg)	Requirement (mg/day)		Reference Wt. (kg)	Requirement (mg/day)	Reference Wt. (kg)	Requirement (mg/day)
6-12 mo	9	0.84	6-12 mo	9	0.84	9	0.84
1-3 year	12	0.83	1-3 year	13	0.74	12	0.53
3-6 year	17	0.97	4-8 year	22	1.20	21	0.83
6-10 year		1.12		25			
10-12 year	35	1.40	8-13 year	40	2.12	38	1.53
12-15 year				48	1.82		
15-18 year M	64	1.97	14-18 year M	64	3.37	64	2.52
15-18 year F	55	1.54	14-18 year F	57	3.02	56	1.98
Pregnancy	-	2.27	Pregnancy*	-	4.1-5.0	-	2.68
Lactation	-	2.89	Lactation*	-	3.8-4.5	-	2.98

***Different stages of pregnancy/lactation.**

2.9. General Assessment of Zinc Status

WHO, UNICEF, and IZiNCG are recommend indicators to assess zinc status and epidemiological criteria have to consider zinc nutrition as a public health concern.

1. If >20% of the population are have low serum zinc
2. If more that 25% of the population has low dietary zinc intake.
3. Prevalence of stunting is > 20% in a given population [43-46].

2.9.1. Biochemical Indicators

Since the use of zinc for human health was recognized, there has been tremendous effort to be carried out to identify and validate indices of zinc levels. However, there has been limited success. So far there is lack of single specific and sensitive biochemical index that shows the whole spectrum of zinc levels from low to excess. However, existed biochemical indicators include serum, erythrocyte, leukocyte, urinary and hair zinc concentrations (10).

Serum zinc is the most frequently used biomarker. Several studies have confirmed its importance for assessing zinc levels at population status. However, at individual level it may not detect marginal deficiency as serum zinc is homestatistically controlled. Erythrocyte zinc is an alternative measurement to indicate zinc concentration status but it does not show short time variations 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 varies at extreme deficiency or high dose consumption states. Hair zinc concentration reflects chronic deficiency when protein energy malnutrition is absent. However, it is not helpful in determining short-term of zinc deficiency [2, 10].

2.9.2. Dietary Intake Method of Zinc Assessment

The assessment of zinc by using dietary intakes in the population is important because inadequate dietary intake of zinc is most likely lead to zinc deficiency.

There are quantitative ways to assessing usual dietary intakes of individuals: weighed food records, recalls, and semi-quantitative food frequency questioners. Of these, food weighed records and recalls are designed to measure the quantity of each food consumed over a one-day period. By contrast, food frequency questionnaire (FFQ) obtains retrospective information on the pattern of food consumption during a longer time period, and sometimes on the usual intakes of certain nutrients [43-45].

Weighed food records completed by trained research assistant in households have been used to collect reliable quantitative data on dietary intakes .This method is more time taking and costly, it has higher respondent burden than other methods, and may increase the lively hood that respondents change their dietary intakes during record period; this may be leads to erroneous outcome. However, weighed food records are the most accurate methods of determining actual consumed during the recording period [44, 45].

Dietary recalls important to estimating zinc consumed among illiterates. It is suitable where diets are not very diverse and dominantly consumed plant food sources. Although some of its accuracy recalls method is compromised by their implementation, and it is easy, faster, and less cost than weighed food records [43, 45].

2.9.3. Prevalence of stunting (clinical assessment)

Prevalence of clinical impacts of zinc deficiency (e.g. vomiting, diarrhea, stunting) has been established, both by experimental animals and by human intervention trials, that show correlation between zinc deficiency and growth-retardation. The response to zinc supplementation were significantly greater in those studies that enrolled subjects with pre-existing nutritional stunting or underweight, defined respectively as height- for- age or weight-for-age z-scores < -2 in relation to international reference data. On the other hands, no significant impact of zinc supplementation of children were non-stunted .These out comes imply that children with low height-for-age or weight-for-age are likely having zinc deficiency. In addition to this it indicate the national prevalence of stunting or underweight for children those ages less than 5 years which in turn used as indirect indicators of risk of zinc deficiency status of a population. Therefore, this method is the indirect method of assessing the risk of zinc deficiency among population. The WHO takes into account if national stunting rates of$\geq$20% to be a level of public health problems [22, 43, and 46].Estimated country-specific prevalence of inadequate zinc intake is indicated in figure 1 below.

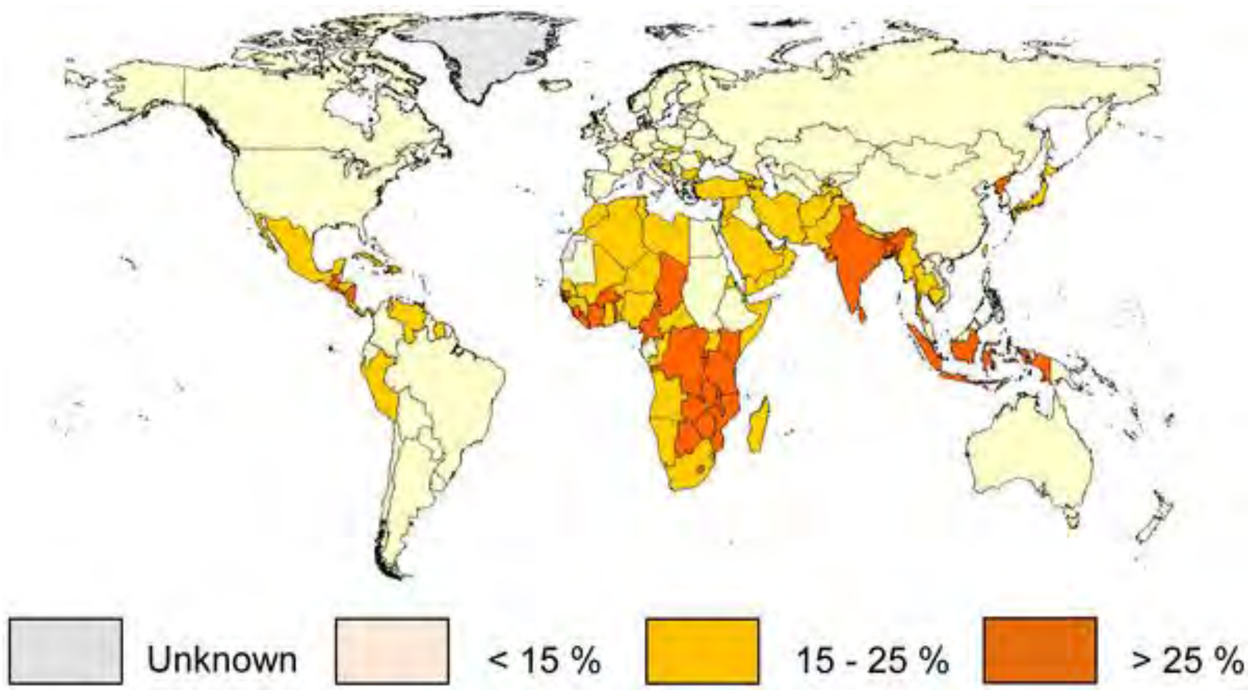

Figure 1 Estimated country-specific prevalence of inadequate zinc intake. (Source: Wessells et al. 2012)

Table3. Regional means (± SD) for national data on daily per capita energy, zinc, phytate and absorbable zinc contents of the national food supply and estimated prevalence of inadequate zinc intake in the population, years 2003–2007. (Source: Wessells et al. 2012)

VARIABLE	High-Income	South AndTropical L America	China	Central and Eastern Europe	Central and Andean L. America and Carib	Central Asia, North Africa and middle East	East and south east Asia and pacific	Sub Saharan Africa	South Asia	Global
Number of countries	30	5	3	20	27	28	21	48	6	188
Population (millions)	937.2	249.4	41337.7	330.1	301.4	481.4	606.8	757.8	1495.6	6497.5
Energy (kcal)	342±323	3031±88	2905±65	3286±175	2836±468	3089±359	2585±170	2351±345	2281±31	2776±472
%of energy from ASF	27.6±5.1	23.2±2.8	21.0±1.2	23.1±2.9	16.3±3.8	11.9±4.3	10.1±4.2	6.8±5.0	9.0±4.0	15.8±8.4
Zinc (mg)	13.2±1.3	12.3±0.9	13.6±0.5	11.7±1.1	10.7±3.1	13.9±3	8.9±1.3	8.4±1.7	9.8±0.7	11.4±2.5
Zinc density (mg/100kcal)	3.9±0.3	4.1±0.3	4.7±0.1	3.6±0.3	3.7±0.6	4.5±0.8	3.4±0.4	3.6±0.5	4.3±0.3	4.1±0.6
% of zinc from ASF	59.7±8.5	59.7±7.2	49.2±2.3	51.8±4.2	39.7±9.7	23.7±11.1	28.0±10.7	19.1±11.6	11.5±4.9	34.8±20.0
Phatate (mg)	1173±173	1170±245	1456±18	1199±210	1887±929	2776±1089	1440±165	1782±499	2559±226	1730±649
Phatate :zinc molar ratio	9.0±2.2	9.6±2.3	10.6±0.8	10.1±1.3	16.6±4.6	19.1±4.5	16.4±3.1	21.3±4.4	22.8±1.1	15.8±6.2
Est. fractional absorption	0.25±0.01	0.26±0.01	0.23±0.0	0.27±0.02	0.26±0.07	0.19±0.05	0.28±0.02	0.27±0.04	0.23±0.02	0.24±0.04
Absorbable	3.32±	3.21±0	3.17±0	3.10±0.	2.53±0.	2.56±0	2.46±0.	2.17±0.	2.19±0	2.71±0.51

zinc (mg)	0.28	.32±	.09	13	21	.24	26	28	.05	
% mean physiological requirement	160.8 ±14.7	164.3± 15.5	155.4± 4.7	149.2± 6.7	133.0± 9.7	133.4± 12.2	127.3± 14.6	123.04 ±14.5	115.7± 4.1	138.0±20.
Est. % of pop. With inadequate zinc intake	7.5±4.1	6.4±1.8	7.8±2.1	9.6±2.4	17.0±5.9	17.1±5.4	22.1±10.0	25.6±12.2	29.6±3.6	17.3±11.1

3. Materials and Methods

3.1. Study Design and period: A cross-sectional study from July-August, 2016

3.2. Study Area: The study was conducted at Kolfe and Mikililand Health Centers in Kolfe Keranio Sub-city, in the capital Addis Ababa.Kolfe and Mikilland Health Centers are service rendering health centers that provide health service to over 62400and49, 000people, respectively. Addis Ababa lies on the average at 2,355 meters above sea level and over 3,627,934 as 2007, population reside in this administrative city [47].Addis Ababa is located at 8º58'N,38º47'E,mean annual temperature is 15.9^{0}c and total annual rain fall averages 1089mm per year. The map of Addis Ababa is shown in figure 2 below.

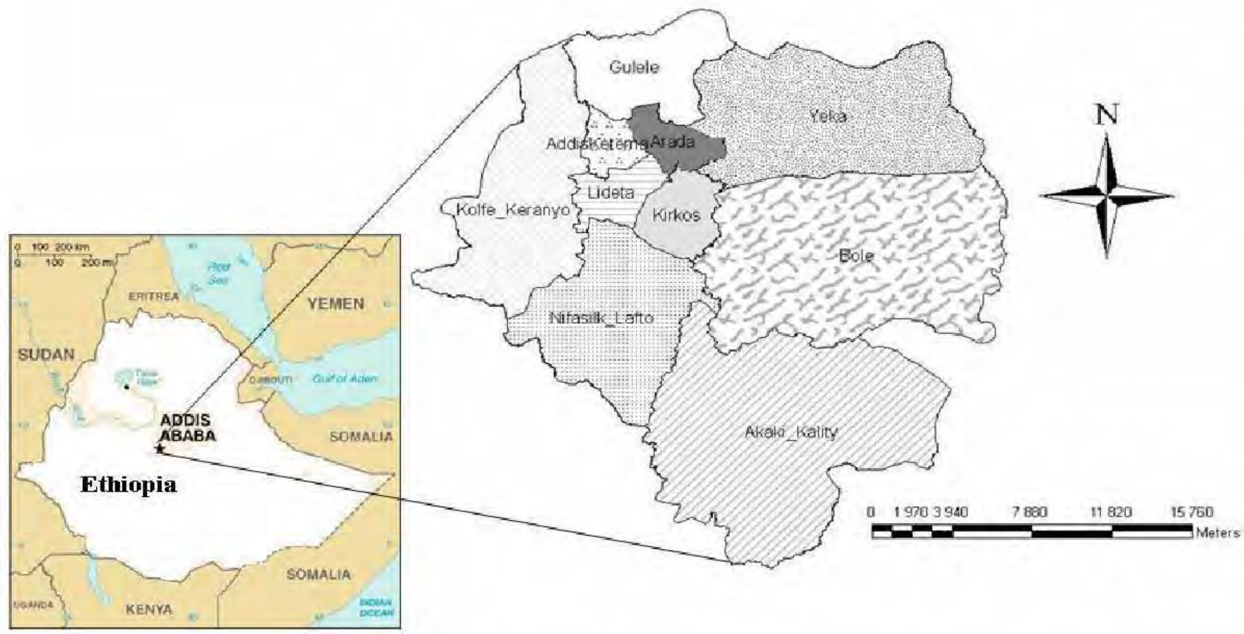

Figure 2 Map of Addis Ababa (source: https://www.researchgate.net/publication/281460707)[48]

3.3. Source Population and Study Population: all pregnant women and pregnant women attending antenatal care in the health centers

3.4. Inclusion Criteria: all pregnant women attending antenatal care except those that are critically ill.

3.5. Study Variables

3.5.1. Dependent Variable: Serum zinc levels

3.5.2.Independent variables: maternal age, gestational trimester ,maternal education, maternal employment, parity, dietary diversity score, consumption of animal source foods, type of staple diet, consumption of coffee, history of iron-folate supplementation, morbidity, and CRP status.

3.6. Sample Size Determination

Sample size was calculated using single proportion sample size calculation formula with inputs of 95% confidence level, (Z=1.96), 5% margin of error, 57% expected prevalence of zinc deficiency [9, 49].

$n=Z^2p.q/d^2$,p=0.574

$n= (1.96)^2(.574) (.426)/(0.05)^2$

=376

Assuminga10% non-response rate hence **413** samples were taken.

3.7. Sampling Method

Pregnant women attending antenatal care at two health centers, Kolfe and Mikililand, in Kolfe Keraniyo Sub city were asked to participate in the study. Both the city and health centers were selected purposefully for logistic reason.

Predetermined sample sizes of 413 were sampled by random sampling from pregnant women attending ANC. Health Centers at the time of study run five days per week, and on average used to select 16 respondents every week. This process was repeated daily for approximately 25 working days until the required sample size of 413 pregnant women were obtained.

3.8. Data Collection Method

3.8.1. Socio-demography

Information regarding socio-demography, feeding, and health were collected by applying interview technique using structured questionnaire (annex 1) which was taken from standard demographic and health survey (DHS) questionnaire with minimal modifications. The part of the questionnaire used to assess the Dietary Diversity (DD) was adopted form Food and Agriculture organization (FAO guide for Individual Dietary Diversity Score (IDDS) [10]. The tool was developed in English and later translated and finalized in Amharic.

IDDS quantifies based on one day 24 hours recall method. Respondents were asked whether they had taken any food form several listed food in previous day of the survey. Accordingly, the Dietary Diversity Score (DDS) was computed out of 9.Pregnant Women with DDS≥6 have high DDS, DDS=4 or5 have medium DDS, and DDS≤3 have low DDS [10].

3.8.2. Blood Sample Collection and Preparation

Blood collection was done by an experienced laboratory technician and follow correct procedure to avoid zinc contamination. About 5ml of blood was collected from antecubital vein using plain and closed SARSTEDT Monovette®blood collection system and SARSTEDT®butterfly stainless steel needles. After sample collection, the blood was allowed to clot for 20 minutes in a closed ice box and centrifuged at 3000revoultion per minute (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. The same day they were stored frozen at -20^0c.At the end of the survey the samples were transported in ice box to the Ethiopian Public Health Institute (EPHI) laboratory and kept frozen at -80^0cuntil analysis.

3.9. Laboratory Analysis

3.9.1. Serum Zinc: Serum zinc was determined within one months of sample collection. The analysis was done at laboratory of Center for Food Science and Nutrition, Addis Ababa University by experienced laboratory technician. The PI was actively involved in sample preparation. The analysis was made using Shimatzn SpectrAA®Flame Atomic Absorption Spectrometers (AAS) in accordance with the manufacturer's operating procedure. Samples were analyzed at a wave length of 213.9nm, with lamp current of 5mA and slit width of 1nm.Intially; standards of 0, 0.1, 0.2, 0.3, and 0.4 PPM were prepared by diluting 1000ppm zinc standard in appropriate volume of 6%butanol.Then the standards were used for calibration. The samples were prepared by diluting well vortexes 200µl serum samples in 2 ml of 6%butanol.After the AAS was optimized, the zinc concentration of each sample was read. Samples were analyzed in a batch of 25.

A control sample with known concentration and multiple blank samples were analyzed 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. Zinc deficiency was defined as a serum zinc level of less than 56 µg/dl during the first trimester, or less than 50µg/dl during the second or third trimester [1].

3.9.2. Hemoglobin:

Hemoglobin level was determined using hematological analyzer (Cell Dyn 1800, PD, USA) machine. Anemia was defined as a hemoglobin level of (at sea level)less than 11.0 g/dl during the first or the third trimester or less than 10.5 g/dl during the second trimester (1).According to the formula recommended by Center for Disease Prevention and Control(CDC), hemoglobin values were adjusted for altitude[1].

3.9.3. C-reactive protein (CRP):

Serum CRP was quantified by particle enhance turbid metric assay with clinic chemistry analyzer Cobas Integra in which human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate was determined turbid metrically at 552nm [20].

3.10. Statistical Analysis

Data entry, screening and analysis were done using SPSS software version 20. Descriptive analysis such as mean, frequency, and percentage was computed. Independent t-test used to compare mean of serum levels of elevated CRP and normal, anemic and not, time of blood collection and one-way Analysis of Variance (ANOVA) used to compare mean of serum zinc levels across categories of independent variables like trimmers ,education, age, parity, DDS,. Normal distribution of data was checked using Kolmogorov-Smirnov.

Logistic and linear regression analyses were used to study the relation between dependent and independent variables. Independent variables which significantly associated 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, homosecedasticity and absence of multicollinearity) were checked to be satisfied.

3.11. Ethical Considerations

The study was conducted following standard ethical guidelines for biomedical research involving human subjects. Ethical clearance was obtained from the institutional review board (IRB) of Addis Ababa University (AAU).In addition, permission was obtained from Addis Ababa City health bureau. Participation of the study subjects was entirely on voluntary basis. Written informed consent was obtained from the study subjects (Annex3). Written consent was obtained after the purpose of the study, the rights of the participants, potential benefits and harms of the study were thoroughly and privately communicated.

4. Results

4.1. Socio-demographic Information

A total of 413 pregnant women were recruited and 403 participated in the study, giving a response rate of 97.6%.The mean age of the respondents was 25.7 (±4.6) years. Nearly three-fourth of study participants (71%) were in grades 1-10. Almost all (98%) were house wives. About one-fifth 21.8% of study participants were employed. The socio-demographic characteristics of participants' are depicted in the table 4 below.

Table 4Socio-demographic characteristics of study subjects, in Selected Health Centers in Addis Ababa, Ethiopia, August201

Variable	n(%)
Age(Y)	
15-24	161(40)
25-34	217(53.8)
>34	25(6.2)
Educational Status	
Illiterate	53,(13.2)
1-6	130(32.2)
7-10	156(38.7)
>10	64(15.9)
Marital Status	
Married	395(98)
Others	8(2)
Ethnicity	
Gurage	184(45.7)
Oromo	72(17.9)
Tgrian	10(2.5)
Amhara	81(20.1)
Others	56(13.9)
Language	
Amharic	160(39.7)
Oromifa	64(15.9)
Tigregna	3(0.9)
Guragegna	135(33.5)

Others	41(10.2)
Religion	
Muslim	188(46.7)
Orthodox	177(43.9)
Protestant	33(8.2)
Others	5(1.2)
Job of Respondents	
Non-employed	315(78.2)
Employed	88(21.8)
Respondent family monthly cost on food	
<500	84(20.8)
500-100	175(43.4)
1100-1500	62(15.4)
>1500	84(20.3)

4.2. Prevalence of Zinc Deficiency

Zinc deficiency was observed in 159(39.5%) pregnant women studied. Study participants at first, second and third trimester had zinc deficiency prevalence of 42.2%, 41.1%and 43.3%, respectively. The mean serum zinc concentration of the study participants was 63.68(±27.3)µg/dl. The individual values were in the range of 21.0 to 194.7µg/dl.

4.3. Correlates of zinc deficiency

4.3.1. Zinc Deficiency and Socio-demographic Factors

More than half of the study subjects, 217(53.8%) were in 25-34years of age and the majority of the pregnant women, 378(93.8%) were less than thirty five years of age. The mean zinc level of study participants in 15-24, 25-34, and ≥35 years of age were66.2 (±27.7), 61.7(±27.4), and63.4(±21.6)µg/dl, respectively (p=0.281).About two-fifth study subjects, 156(38.7%) were in grades 6-10 and the pregnant women greater than grade 10 were, 64(8.2%).The mean zinc concentration of study participants in grades >10,7-10,1-6, and illiterates were67±29.0;61.3±26.1,60.8±23,and73.4±33.3µg/dl,respectively.Serum zinc concentration was significantly different by education status (p=0.04).Surprisingly ,illiterate mothers had significantly higher serum zinc concentration than mothers who attend primary education(p=0.013) and secondary education (p=0.012). But the difference in serum zinc concentration of illiterate and mothers above grade ten was statistically not significant.

Monthly family cost on food and mean zinc status of study participants, were positively associated with zinc status, <500(57.1±20.1), 500-1000(62.2±26.2), 1100-1500(62.9±24.8) &>1500(74.0±34.0) (birr, µg/dl), respectively (F=5.99, p=0.001).

4.3.2. Zinc Deficiency and Reproductive Factors

One third of study participants, 135(33.5%) were in first trimester at a time of data collection. The parity of the study participants was ranging between 0 and 8.Nearly half, 193(47.9%) of study participants were null parity. About half, 192(47.6%) of study participants was in the second trimester of pregnancy. The mean zinc level of first, second, and third trimester were64.87 (±27.5), 60.72(±26.1), and 69(±28.6)µg/dl, respectively. The mean zinc level by parity category was not significantly different (p=0.167).Reproductive characteristics of the study participants are shown in 5below.

Table 5 Reproductive health factors among pregnant women Attending ANC in Selected Health Centers in Addis Ababa, Ethiopia, August2016

Characteristics		(n)	(%)
Parity	0	193	47.9
	1-2	172	42.7
	3-4	31	7.7
	≥5	7	1.7

Trimester	First	135	33.5
	Second	192	47.6
	Third	76	18.9

4.3.3. Zinc Deficiency and Clinical Factors

More than half of the serum samples (64.3%) were collected during the morning time. Zinc levels for samples collected in the morning and afternoon were 64.2(±27.4) and 62.8(±27) µg/dl, respectively (p=0.63).About one-fifth of the study participants (n=82) had elevated CRP concentration (≥5mg/dl).The mean zinc concentration of participants with normal (<5mg/dl) and elevated CRP were 60.7(±26) and 64.4(±27.5) µg/dl, respectively. Based on logistic regression model participants with elevated CRP had higher risk of zinc deficiency (p=0.001).The risk of zinc deficiency for pregnant women with elevated C-reactive protein was two and half times higher as compared to pregnant women with normal C-reactive protein [AOR=2.48; 95%(CI 1.45, 4.24)].

About one in ten (10.1%) of pregnant mothers were anemic. In the present study, hemoglobin was significantly correlated with serum zinc (r=0.208; p<0.001).The mean zinc concentration for anemic pregnant women and non-anemic were 55.1(±22.1) and 64.6(±27.6) µg/dl, respectively (p=0.03). Study participants who took iron-folate supplements had significantly higher hemoglobin concentration compared to participants who didn't take the supplement (12.4±0.9vs9.7±1.2; p<0.001). Of all respondents, 53(13.2%) took iron-folate supplement (daily dose of 150 mg ferrous sulphate and 500µg folate) at least once in the preceding of the survey. The mean zinc levels for pregnant women took iron-folate supplementation and those do not took iron-folate supplementation were, 69.3(±28.5) and 62.8(±27) µg/dl, respectively (p=0.10).

About one-third, 129(32%) of the study participants were infected with one or more diseases. Morbidity during current pregnancy was significantly associated with zinc status of pregnant women. Based on the logistic model the risk of zinc deficiency was two times, [AOR = 1.98; 95% CI (1.25, 3.14)] higher among pregnant women who had morbidity than those who had not morbidity during pregnancy (p=0.04).Clinical characteristics of the study subjects are shown in the following table6.

Table 6 Clinical factors among pregnant women attending ANC in Selected Health Centers in Addis Ababa, Ethiopia, August2016

Characteristics		n	%
CRP(mg/dl)	≥5	82	20.3
	<5	321	79.7
Anemic	No	362	89.8
	Yes	41	10.2
Morbidity during pregnancy	No	274	68.0
	Yes	129	32.0
Iron-folate supplementation	No	350	86.8
	Yes	53	13.2
Time of blood sample collection	Morning	259	64.3
	Afternoon	144	35.7

4.3.4. Zinc Deficiency and Dietary Characteristics

The dominant diets for the vast majority, 391(97%), of the study participants was Teff .Results of study revealed that more than half, 216(53.6%) of the study participants were drinking coffee after a meal. The mean zinc levels for those drinking coffee and not consume coffeewere57.3 (±26) and 71(±27) µg/dl, respectively. Using logistic model, significant associations were observed between zinc status and coffee intake (P= 0.001).Compared to pregnant women who did not consume coffee, the risk of zinc deficiency was 2.12 times higher among those who consumed coffee in the reference period [AOR = 2.12; 95 % CI (1.39, 3.42)].

The dietary diversity (DD) level was assessed using 24 h recall method. The mean dietary diversity (DD) of the study participants was 5.48(±1.35), ranging between 1 to 8.The majority of the study participants, 372(92.3%) had medium to high DD score (>3 food groups).45.9 %(189) of study participants had high DDS. The mean serum zinc concentrations of dietary diversity score of low, medium, and high of the study participants were 78(±31.5), 64.7(±28.7), and 60.1(±23.9) µg/dl, respectively. However, groups with low dietary diversity had higher mean serum zinc than other groups (p=0.02).The highest consumed food groups by the study participants were starchy staples (99.3%), and lipids (97.5%) (Fig3). About two-fifth (40.2%) of pregnant women were consumed diet of animal origin. The mean zinc levels of study participants that consumed animal food sources and those not consumed were 64.7(±25) and 62.9(±28.60) µg/dl, respectively (p=0.003). The risk of zinc deficiency for pregnant women who did not consume animal source foods was two times higher, as compared to pregnant women who consumed animal source foods in the reference period[AOR = 2.11; 95% CI (1.30, 3.42)]. Dietary diversity of study subjects are shown in table 7 below, food groups of pregnant women are indicated in figure 3 below, dietary characteristics of study subjects are shown in table 8below and also DDS of study subjects are indicated in figure 7 below.

Table7Dietary diversity characteristics among pregnant women attending ANC in Selected Health Centers in Addis Ababa, Ethiopia, August2016

Dietary diversity (DD)	n (%)
Starchy foods	400 (99.3)
Legumes	271(67.2)
Others fruits	122(30.3)
Vitamin A Sources	63(15.6)
Lipids	393(97.5)
Milk and dairy products	62(15.4)
Egg	38(9.4)
Meats	100(24.8)
Fish	3(0.7)

Table8 Nutrition related characteristics among pregnant women attending ANC in Selected Health Centers in Addis Ababa, Ethiopia, August2016

Characteristics		(n)	(%)
Animal source foods	Yes	162	40.2
	No	241	59.8
Dietary diversity score(DDS)	Low	31	7.7
	Medium	189	46.9
	High	183	45.4
Coffee intake	Yes	216	53.6
	NO	187	46.4

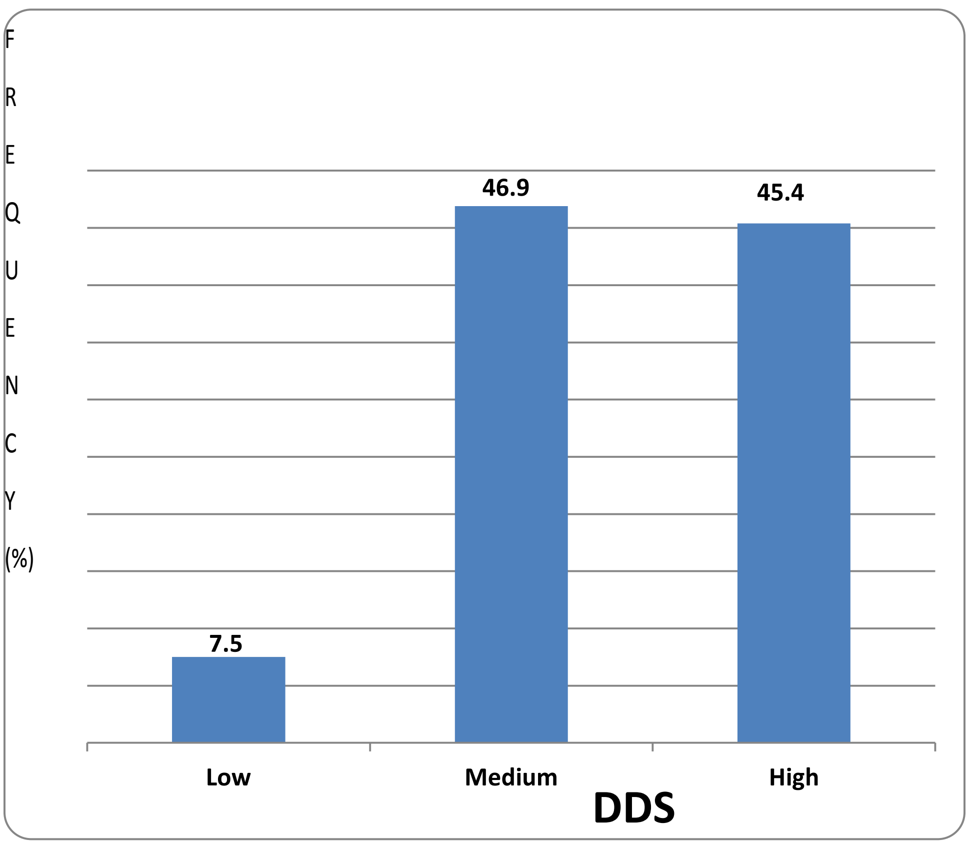

Figure 3 Dietary diversity score of pregnant women attending ANC in Selected Health Centers in Addis Ababa, Ethiopia, August2016

4.4. Factors associated with serum zinc concentration among pregnant women

A multivariable analysis in a form of logistic regression was employed to identify the risk factors of zinc deficiency among pregnant women. Independent variables that turned out to be significant in bivariate regression models were exported to a multivariable regression model for adjustment.

In the bivariate analysis, the risk of zinc deficiency was significantly associated with trimester of pregnancy, morbidity during pregnancy, low hemoglobin level, animal source food, dietary diversity, coffee intake and C-reactive protein. The multivariable logistic regression analysis revealed that morbidity during pregnancy, animal source food; coffee intake and C-reactive protein were predictors of zinc deficiency. Results of bivariate and multivariate analysis of the relationship between potential risk factors for zinc deficiency is shown in table below

Table9Factors associated with zinc deficiency ANC in Addis Ababa, Ethiopia, August2016

Predictors	Zinc Deficiency		COR (95%CI)	AOR (95% CI)	P- values
	Yes	No			
Educational status					
No formal education	14	39	1.67(0.76, 3.69)		
Primary education	49	81	0.99(0.54, 1.84)		
Secondary education	72	84	0.70(0.39, 1.27)		
Certificate and above	24	40	1		
First	57	78	1		
Second	79	113	1.05 (0.67, 1.63)		
Third	23	53	1.68 (0.93, 3.06)		
Morbidity during pregnancy					
No	94	180	1	1	
Yes	65	64	1.95(1.27, 2.98)	1.98 (1.25, 3.14)	0.004
Anemia					
No	138	224	1		
Yes	20	21	1.70(0.89, 3.26)		
Animal food sources					
Yes	54	108	1	1	
No	105	136	1.54 (1.02, 2.34)	2.11 (1.30, 3.42)	0.002
Dietary diversity					
Low	6	25	3.10 (1.21, 7.91)		
Medium	75	114	1.13 (0.75, 1.71)		
High	78	105	1		
Coffee intake					
No	54	133	1	1	
Yes	105	111	2.33 (1.54, 3.52)	2.12(1.39, 3.42)	0.001
C-reactive protein					
Negative	115	206	1	1	

Positive	44	38	2.07 (1.27, 3.39)	2.48(1.45, 4.24)	0.001

5. Discussion

Zinc deficiency is the most wide spread micronutrient deficiency and a common contributor to poor growth, intellectual impairment, prenatal complications, and increased risk of morbidity and mortality. Of greatest concern is the fact that the cycle of zinc deficiency continue across generation with far reaching consequences on the future population [12]. Pregnant women and children under 5 years of age are at high risk of zinc deficiency [3, 12].The major factor associated with the development of zinc deficiency is inadequate intakes of dietary zinc, physiological requirements and mal absorption [2].The present study estimated the prevalence and associated risk factors of zinc deficiency of pregnant women from Addis Ababa.

In the current study, 39.5% of pregnant women had low serum zinc. According to IZiNCG, the risk of deficiency is of public health concern when the prevalence of low serum zinc concentrations is greater than 20% [1].Zinc deficiency prevalence in the present study subjects was lower than available reports from other parts of the country. Study found that 53% of pregnant mothers from rural Southern Ethiopia have low serum zinc [1]. In addition, another study reported 57% lower serum zinc from North West Ethiopia [9]. This is not surprising that less percentage of pregnant mothers in the present study had low serum this due to the fact that, they have relatively better access to diversified foods that can boost zinc concentration [1, 9] and in addition, they have relatively more access to health service, and communication infrastructure and thus may be enabling them to be more knowledgeable about their nutrition. In addition, urban inhabitants have access to information that can positively influence their dietary, sanitation and hygiene habit and then serum zinc concentration [9].Moreover, the higher prevalence of zinc deficiency in the previous studies might also be due to seasonal differences and time gap [12].

In 2004, based on national food supply statistics, IZiNCG estimated that 21.1% of Ethiopian population was at risk of inadequate dietary zinc intake [10].Accordingly, the country was classified in medium-risk category for the zinc deficiency which was lower than zinc deficiency prevalence in the present study (39.5%) this might be due to increase physiological changes during pregnancy that demand additional zinc requirement to accommodate hormonal changes. Therefore, pregnant women are more susceptible to zinc deficiency than others groups of population[3].The existence of low serum zinc status was attributed to four major factors: low intakes of foods of animal origin, morbidity during pregnancy, coffee intake and C-reactive protein (CRP), each of which is discussed below.

Foods of animal origin were significantly associated with serum zinc status of pregnant women. Compared to pregnant women who consumed animal source foods, the risk of zinc deficiency was 2.11(95%CI: 1.30, 3.42, p=0.002) times higher among those who did not consume animal source foods in the reference time. Previous studies conducted in southern Ethiopia [1] and Gondar [9] also similar to the finding. About two-fifth, 162(40.2%) of pregnant women consumed diet of animal origin, which are good sources of bioavailable zinc. This due to the presence of sulfur-containing amino acids (cysteine and methionine) that improve zinc absorption and absence of phytic acid ,a compound that inhibit zinc absorption in animal origin food sources[2, 3].

The study participants were characterized by higher consumption of cereals (99.3%) and legumes (67.2%). Such diets contain high level of phytates that are known to inhibit zinc absorption [2, 3].The large dependency on plants diets (cereals and legumes), which have low zinc absorption and less intake of foods of animal origin, which are a rich source of readily available zinc and that contain protein enhance zinc absorption, this may be one risk factor for zinc deficiency in the study subjects. Cereals and legumes do contribute relatively high amounts of zinc but also contribute importantly to phytate intakes which highly reduce zinc bioavailability. Thus, the high prevalence of zinc deficiency in the present study could be partially be explained by the poor zinc absorption from the plant-based diets. High prevalence of zinc deficiency in the present finding might be also partially explained by low zinc content of Ethiopia soil, as crops harvested locally will have low zinc content and household who depend entirely on locally grown crops will be at risk of inadequate zinc intake [20].

Coffee intake during pregnancy was negatively associated with zinc status. Women who drink coffee after meal had 2.12(95%CI: 1.39-3.42) times higher risk of zinc deficiency compared to their counters. This is due to tannin commonly found within coffee which can potentially inhibit zinc absorption. Parallel finding was reported in Southern Ethiopia [10].

Morbidity during pregnancy was one of the significant factors influencing serum zinc concentration of pregnant women. Pregnant women who experienced morbidity two weeks preceding the survey had 1.98(95%CI: 1.25, 3.14) times higher zinc deficiency as compared to their health counter parts. In current study, 129(32%) of study participants were infected with one or more disease .Of whom more than half, 65(50.38%) were zinc deficient. Various health problems may decrease zinc level and cause zinc deficiency .Previous study conducted in North West Ethiopia also similar with the finding [9]. This might be due to infectious and disorders like gastrointestinal tract, infectious hepatitis, alcoholism, protein-energy mal nutrition, diarrhea, and sickle cell anemia are may cause mal absorption of zinc and decrease the level of the zinc [4, 27].

Study also showed negative relationship between zinc deficiency and C-reactive protein (CRP).Pregnant women with elevated CRP had 2.48(95%CI: 1.45, 4.24) times higher zinc deficiency compared to women whose CRP are normal .High sensitive CRP is an acute phase reactant protein that is secreted to the circulation from the liver as a reaction to the onset of inflammation/acute infection[10].The elevated CRP indicate that the presence of acute inflammation/infection which independently affect serum zinc concentration. The concentration is decreased during acute infection/inflammation probably due to the redistribution of zinc from plasma to tissue [10]. A similar previous study also found significant negative association between zinc concentration and an elevated CRP [10].

This study also indicated positive relationship between zinc deficiency and anemia. Zinc deficiency and anemia tend to occur together. About 40(12.6%) of the subjects had both anemia and zinc deficiency and about half, 20(48.8%) of anemic pregnant women had zinc deficiency. Similarly previous studies also found positive significant association between zinc deficiency and lower hemoglobin [9, 10].As studies are cross-sectional it is not viable to implicate causal inference. However, as zinc is known to take part in multiple metabolic path ways thus the deficiency might have casual role in anemia. This relation between zinc deficiency and anemia might be due to the role of zinc participate in the production of hemoglobin. Therefore, low concentration of zinc likely related to low production of hemoglobin thereby lead to anemia [2].

Based on the data of pregnant women included in US National Health and Nutrition Examination Survey (NHANES),IZiNCG suggested trimester specific cutoffs points for defining prenatal zinc deficiency [10].The suggested point for the first trimester was 56µg/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%µg/dl was recommended [10].However, in the current study a decline in zinc level from first to second trimesters and rise of zinc level from second to third trimesters was observed. The zinc level in the second trimester was significantly lower than that of third trimester. This finding is inconsistent with the recommended cut-off and other previous findings [1, 9]. This might be due to their dietary intake and in addition, due to numbers of samples cause source of variation because that of second (192) and third (76).Moreover this might be due to hemodilution.

The study witness there were negatively significant association between prenatal zinc status and dietary diversity score (DDS).This is against findings of the previous studies[1.9];pregnant women with lower DDS had higher serum zinc than others groups .This might be due to the reason they consumed diets with high DDSs but low content of zinc concentration or low bioavailable zinc to absorption or both that compared to those consumed diets with low DDSs but with high zinc content or easily bioavialable zinc or both high content and bioavailable zinc and/or DDS was derived from 24hour recall which is limited to show the usual intake pattern of study participants.

6. Limitation of the study

- Since the study was facility based and the participants may not represent the general population
- Assessment of dietary intake depends on the 24hour recall method, which may not accurately reflect their past feeding experience

7. Conclusion

Zinc is essential to sustain life and for optimal biological roles. Wide prevalence global zinc deficiency exist, with pregnant women are at the highest risk. Zinc deficiency is commonly attributed to prenatal complications and poor pregnancy outcomes. The study presented the finding of study focusing on determining prevalence of zinc deficiency and socio-demography, reproduction, clinical, and dietary factors that influencing serum zinc levels of pregnant women in Addis Ababa. Pregnant women are most vulnerable than the other groups. The severity and extent of zinc deficiency will determine its consequences. Thus, a need exists to make informed evidence-based strategies to the prevention of zinc deficiency at national level.

The finding of the present study revealed a high prevalence of zinc deficiency (39.5%).Therefore study findings provide evidence for health significance of zinc deficiency in the study area. Morbidity during pregnancy, low intakes of foods of animal origin, coffee intake and elevated C-reactive protein were pertinent risk factor significantly associated with zinc deficiency. Intakes of animal food sources, intake of iron-folate supplementation, monthly family cost on food and hemoglobin concentration were positively associated with zinc concentration but intake of coffee, DDS, education, elevated CRP and presence of morbidity during pregnancy were negatively associated with zinc level concentration. Zinc deficiency and anemia tend to occur together. Religion, ethnicity, parity, trimesters, and age of pregnant women were not significantly associated with zinc levels.

8. Recommendation

- In Ethiopia national level zinc prevalence study should be conducted so as to assess the extent of the problems in vulnerable groups like pregnant women
- Encourage pregnant women to consume more animal food sources
- Promotion of livelihood and socio-economic empowerment of women
- Increase the time gap between meal and intake of coffee
- Encourages diversified feeding practices and promoting and practices the consumption of zinc rich foods by Health and agricultural sectors.
- The existing efforts to improve women's awareness concerning the optimal nutrition prior and during pregnancy should be strengthened through building the capacity health extension workers. Special emphasis should be given in enhancing the nutrition education and counseling skills.
- Create access to health facilities and encourage pregnant women to examine for every health problems faced with and get timely treatment
- Motivate and attract Non-Governmental Organization (NGO) working with micronutrient deficiencies like zinc deficiency by policy makers
- The effect of education and dietary diversity (DD) on zinc levels should be further studied

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10. Annexes

Annex 10.1. Questionnaire (English Version)

Addis Ababa University College of Natural Science

Center for Food Science and Nutrition

Questionnaire for assessment of prevalence of zinc deficiency and its associated risk factors among pregnant women from selected health centers in Addis Ababa, Ethiopia

Questionnaire Code NO……………………………………..

Name of interviewer……………………………Code No…………………..

Respondent's Residence……………………

Date of interview………………..Time started………………Time finished……….

Questionnaire checked……….

		Socio-demographic information		
No	**Questions**	**Choices**		**Remark**
101	Age in years	1.15-24 2.25-34 3.>34		
102	Religion	1.Orthodox 2.Muslim 3.Protestant 4.if other specify		
103	Language	1. Amharigna		

		2. Oromigna 3. Tigrigna 4. Others		
104	Ethnicity	1. Amhara 2. Oromo 3. Tigrian 4. Others		
105	Educational status	1. Illiterate 2. Informal education 3.12th complete 4.above 12		
106	Marital status	1.Married 2.Single 3. Widow 4. Divorced		
107	Parity	1.0 2.1-2 3.3-4 4.>5		
108	Trimester	1.Trimester 1		

		2. Trimester 2 3. Trimester 3		
Socio-economic information				
109	Do you have a job?	0. no 1. yes		
110	If yes, how much do you approximately earn per month in birr?	1 below 7, 00 2. 7, 00- 1,600 3. 1601-3,000 4.above 3,000		
111	Approximately, how much money do you spend on food per month?	1.below 500 2.500-1000 3.1100-1500 4.above 1500		
Morbidity data				
112	Have you suffered any illness during the pregnancy period?	0.no 1.yes		
113	If yes, specify the illness			
Information related house holdfood security				
No	Question	0.no 1.yes		

114	In the past week, did you have reduced meal size because of lack of food in the household?			
115	In the past week, did you have skip meals because of lack of food in the household?			
116	In the past four weeks, did you hungry because of lack of food in the household?			
117	In the past four weeks, did you borrow money to buy food to the household?			
Multi-vitamins supplementation				
118	Did you ever take any multi-vitamin supplementation?	0.no 1.yes		
119	If yes, specify			

Food frequency

No	Food item	Did you ever eat? 0.no 1.yes	Average number of servings

			Per day	Per week
120	Teff			
	Sorghum			
	Millet			
	Berbere			
	Maize			
	Wheat			
	Rice			
	Cassava			
	Sweet potato			
	Potato			
	Bean			
	Pea			
	Ground nut			
	Tomato			
	Carrot			
	Leafy vegetable			
	Eggs			
	Beef			
	Sheep			

	Goat			
	Ox/cow			
	Chicken			
	Fish			
	Milk/milk product			
	Coke			
	Tringo			
	Lemon			
	Orange			
	Banana			
	Papaya			
	Mango			
	Avocado			
	Oils			
	Fats			
	Honey			
	Sugar			
	Coffee			
	Tea			
	Sweet beverages			

	Alcohol			

Biochemical analysis

Questionnaire Code no.…………..

Name of the interviewer…………………………………..Code No……………….. Date of Birth…………………………..Age……………

Date of Sampling………………….Time of Sampling………………… Temperature……………………….

Name of Laboratory Technician……………………………Signature………………

(For official use only)

Sample code	Serum zinc(mg/ml)	C-reactive protein

Annex 10.2: Consent Form (English)

Title: Prevalence of zinc deficiency and associated risk factors among pregnant women from selected health centers in Addis Ababa, Ethiopia

Principal Investigator: Kebebew Regassa

Introduction

Zinc is a micronutrient found in our food that helps our body to make energy, normal function of brain, and recovery from illness. The newly synthesized fetal and maternal tissue during pregnancy causes additional physiological requirements for zinc. If zinc intake low it can causes zinc deficiency which will lead to complications especially during pregnancy for fetus and mother.

We came from the Center for Food Science and Nutrition, Addis Ababa University. In the present MSc research project, we would like to evaluate dietary, demography, economic, multi-vitamin supplementation of pregnant women and associate this to their serum zinc status.

Procedures

If you agree to participate, we will be asking you some basic information, collect data on the type and amount of food you consume during the day, and collect blood (<5ml) by health professionals.

Risks

The risks will be minimal and not more than what is routinely encountered during blood collection in health care centers.

Benefits

The finding from this study may contribute to knowledge on maternal child care in study area and any other with similar characteristics. The finding may be used in formulating policies that will help improve nutrition status of pregnant women of all communities in the country.

Cost

There is no cost to you for participating

Compensation

There will be no financial compensation for participating but you will be able to know your serum zinc status.

Participant Rights

If you said things that are not clear to you, you may ask without hesitation and I will answer. You may feel free and ask questions. Your participation in the study is just is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. If you do not agree to participate, you can say 'no' without worries.

Confidentiality

Tests results and any information about you will be kept private. Only the research team will have access to your information. When I write a report, every one's information about you or any together individual cannot be seen. You will be identified with random numbers on serum vials. A list with the names and numbers will be kept in a private, locked file cabinet.

Persons to contact:

If you have any questions, you can ask at any time. If you have additional questions about the study, you may contact:

Dawd Gashu (PhD)

Assistant Professor

Center for Food Science and Nutrition

Addis Ababa University

Email:dawdgashu@yahoo.com

If you agree to participate in the study, please sign at the space indicated below.

Thank you for your cooperation.

The study has been explained to me and my questions have been answered to my satisfaction.

I agree to participate in this study.

…………………………… …………………………….
……………

Signature Name Date

…………………………. …………………………… ………………

Signature of study representative Name Date

Annex10.3Questinnarie (Amharic)

የ ዚ ን ክ ማነ ስ ሰ ር ጭት ና ተ ያ ያ ዥምክ ን ያ ቶ ች በ ነ ፍ ሰ ጡር ሴቶ ች በ ነ ፍ ሰ ጡር ሴቶ ች በ ኮ ልፌ፣ ሰ ላ ም፣ ሚ ኪለ ላ ን ድና በ ሻ ጎ ለ ጤና ጣቢያ በ አ ዲስ አ በ ባ ከ ተ ማኢት ዮ ጲ ያ

የ ተ ሳ ታፊ መለ ያ ቁ ጥ ር ---

የ ጠያ ቂ ዉስ ም----------------------------መለ ያ ቁ ጥር -----------------

የ ተጠያ ቂ ዉመኖ ሪ ያ ቦ ታ--

የ መጠይቁ ቀ ን ና ሳ ዓ ት --

መጠይቁ ያ ተረ ጋ ገ ጠበ ት ሰ ዉ---

መጠይቅ

		ሥነ ህ ዝ ብመረ ጃ		
ተ.ቁ	ጥያ ቄ	**ምር ጫ**	**ሃ ሳ ብ**	
01	ዕ ድሜዝ ዓ መት	1.15-24 2.25-34 3.>34		
02	ሐይ ማኖ ት	1.ኦ ር ቶ ዶ ክ 2.መስ ሊ ም 3.ፕሮ ቴ ስ ታን ት 4.ሌላ ከ ሆነ ግ ለ ፅ		
03	ቋ ን ቋ	1. አ ማረ ኛ 2. ኦ ሮ ሞኛ 3. ትግ ረ ኛ 4. ሌላ		
04	ብሔር	1. አ ማረ ኛ 2. ኦ ሮ መኛ		

		3. ትግረኛ 4. ሌላ		
05	የት/ትደረጃ	1. ያልተማረ 2. መደበኛያልሆነ ት/ት 3.የጨረሰ 4. ከ 12በላይ		
06	የጋብቻሁኔታ	1.ያገባች 2.ያላገባች 3.(ባሏየ ሞተባት) 4. (ፊት)		
07	ልጆች	1. 0 2)1-2 3.3-4 4) ›4		
08	የእርግዝናወቅት?	1. ትራይሜስተር 1ኛ 2. ትራይሜስተር 2ኛ 3. ትራይሜስተር 3ኛ		

ኀበራዊምጣኔ ነ ከመረጃ

09	ሥራችሎታ	0. የለኝም 1. አዎ		
10	በወርበግምትስንትያገኛ	1 7, 00በታች		

	ሉ?	2. 7, 00- 1,600 3. 1601-3,000 4.ከ 3,000በ ላ ይ		
11	በ ግ ምት ከ ሚያ ገ ኙት ገ ቢው ስ ጥ በ ወር ለ ምግ ብየ ሚያ ው ሉት	1. 500በ ታች 2.500-1000 3.1100-1500 4.above 1500		

ቤና ን በ ተ መለ ከ ተ የ መረ ጃ ሁኔ ታ

12	በ እ ር ግ ዝና ወቅ ት አ ሞት ያ ውቃል ?አ ሞት የ ሚያ ወቅ ከ ሆ ን ህ መመሲገ ለ ፅ	0. አ ይደ ለ ም 1.አ ዎ		
13	የ ሚያ ወቅ ከ ሆነ ህ መመሲገ ለ ፅ ?			

ቤተ ሰ ብየ ምግ ብዋ ስ ት ና የ ተ መለ ከ ተ መረ ጃ

No	ጥያ ቄ	0. አ ይደ ለ ም 1.አ ዎ		
14	ባ ለ ፈ ወሳ ምን ት ወስ ጥ በ ም ግ ብእ ጥረ ት ምክ ን ያ ት በ ሚ ት በ ይ ውምግ ብቀ ን ሰ ሽነ በ ር ?			
15	ባ ለ ፈ ወሳ ምን ት ወሰ ጥ በ ም ግ ብእ ጥረ ት ምክ ን ያ ት			

16	ባለፈው 4 ሳምንትውስጥበቤትምግብ ባለመኖሩተርበውኅ በር?			
17	ባለፊው4ሣምንትውስጥለቤተሰብምለምገ ብብለንገን ዘብተበድረውኅ በር?			

20.የ ምግብድግግሞሽ

·.ቁ	የ ምግብዝርዝር	በልተውያውቃሉ 1. አዎ 2. አይደለም	በአማካይ	
			የቀን	የሳምንት
	ጤፍ			
	ዘንጋዳ			
	ዳጉሣ			
	በርበሬ			
	በቆሎ			
	ስንዴ			
	ሩዝ			
	ስኳርድንች			
	ድንች			
	ባቄላ			
	አተር			

	ቲማቲም			
	ካሮት			
	ቅጠላ ቅጠል			
	እንቁላል			
	የአሣደሥጋ			
	በግ			
	ፍየል			
	የበሬ/የላምስጋ			
	የዶሮስጋ			
	ዓሣ			
	ወተትናየወተትተዋፆኦ			
	ትርንጐ			
	ሎሚ			
	ብርቱካን			
	መዝ			
	ፖፖያ			
	ማንጐ			
	አቫካዶ			
	ዘይት			
	ቅባት			

	ማር			
	ስ ኳር			
	ቡና			
	ሻ ይ			
	ጣፋጭ ፈሳ ሽ			
	መጠጥ			

Annex10.4 የ ጥና ትፈቃድፎር ም (Consent form of Amharic)

የ ዚን ክ ማነ ስ ሥር ጭት ና ተ ያ ያ ዠምክ ን ያ ቶ ች በ ነ ፍሰ ጡር ሴቶ ች በ ኮ ልፈ፣ ሚኪለ ላ ን ድጤና ጣቢያ በ አ ዲ ስ አ በ ባ ከ ተ ማኢት ዮ ጵ ያ

ዋ ና ተ መራ ማሪ ክ በ በ ውረ ጋ ሣ

ተ ቋ ም፡ አ ዲስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ የ ምግ ብሳ ይን ስ ና ኒ ውት ሬ ሽ ን ማዕ ከ ል

መግ ቢያ

ዝን ክ ከ ምግ ብየ ሚገ ና ኙን ጥረ ነ ገ ር ሲሆን ለ ሰ ውነ ታችን የ ሚሰ ጠውጥቅ ምጉ ልበ ትእ ን ድና ገ ኙይረ ዳል ፣ የ አ ስ ተ ሳ ሰ ብና የ መማር ክ ህ ሎት በ እ ጅጉ የ ሚጨምር ና የ ሰ ዎችን የ በ ሽ ታመከ ላ ከ ልአ ቅ ምየ ሚያ ጎ ለ ብ ትነ ው፡ ፡ በ እ ር ግ ዝና ጊ ዜየ ተፈ ጠረ ውፅ ን ስ ተ ጨማሪ የ ዝን ክ መጠን ይፈ ል ጋ ል፡ ፡ ስ ለ ዚ ህ እ ና ት የ ዋ በ ዚ ህ ጊ ዜ በ ቂ ዚን ክ ካ ላ ገ ኘችለ ተለ ያ የ የ ጤና እ ክ ል ልት ዳ ረ ግ ትችላ ለ ች፡ ፡
እ ኔ የ መጣሁት ከ አ ዲስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ የ ምግ ብሳ ይን ስ ና ኒ ውት ሬ ሽ ን ማዕ ከ ል ሲሆን በ አ ሁኑ ኤምኤስ ሲ ጥና ትስ ራለ ማጥና ትየ ፈለ ኩት ስ ለ ስ ለ ዚን ክ ማነ ስ ስ ር ጭት እ ና ስ ለ ምግ ብ፣ ስ ነ ህ ዝብመረ ጃ፣ ስ ለ ኢ ኮ ኖ ሚ፣ በ ነ ፍሰ ጡር እ ና ቶ ችላ ይበ ዚን ክ ማነ ስ ላ ይስ ላ ላ ቸውተ ፅ ዕ ኖ ነ ው፡ ፡

መመሪያዎች

ለመሳተፍከተስማሙእኛየምንጠይቀውስለሚበሉትየምግብዓይነትና መጠንናከ 5 ሚሚያነሰየደምናሙና በሠለጠነ የጤና ባለሙያ ስለመውሰድነው፡፡

ስጋቶች

ጥናቱየ ወሰድነ ውን ደምመመር መር ብቻየ ሚያካትትበመሆኑ በዚህ ጥና ትመሳተፍምን ምአይነ ትጉዳትላ ይአያስከተልም፡፡

ጥቅሞች

ዚንክለነ ፍሰጡር ናለሚወልዱትህፃ ናትበጣምጠቃሚከ ሚበሉትን ጥረነ ገ ሮችይመደባል፡፡ይህን ጥረ ነ ገ ርየ ሰዎችየ አስተሳሰብናየ መማር ክህሎትበእጅጉየ ሚጨምር ናነ ፍሰጡር ሴቶችን ከበሽታይከላከላል፡፡ስለዚህየ ጥና ቱውጤትየ ነ ፍሰጡር ሴቶችን ኒ ውትሬሽን ባጠቃላይበክ ልልምሆነ በሀገ ር ደረጃትክክለኛፖሊሲለመቅረጽናለመተግበር ይረዳል፡፡

ወጭ

በመሳተፍዎምን ምአይነ ትወጭየ ለብዎትም፡፡

ካሣ

በመሳተፍዎምን ምአይነ ትየ ማካካሻካሣአያገ ኙም፡፡

የተሳታፊዎችመብቶች

ግልፅያልሆኑነ ገ ሮችያልኩእን ደሆን ያለምን ምማመን ታትሊጠይቁኝይችላሉ፡፡እኔ ምመልስእሰጣለ ሁ፡፡ተሳትፎሙሉበሙሉበእርስዎፍቃደኝነ ትላይየ ተመሰረ ተሲሆን መወሰን የ ርሶ ፈን ታነ ው፡፡ለመ ሳተፍካልተስማምን ምአይነ ትቅጣትየ ለውም፡፡የ ጤና ጣቢያውእና የ ጤና ባለሙያዎቹየ ተለመደውን የ ጤና አገ ልግሎትይሰጥዎታል፡፡

ሚስጥራዊነ ት

የ እርስዎየ ምር መራውጤትምሆነ መረ ጃሚስጢራዊነ ቱየ ተጠበቀነ ው፡፡ጥና ቱን የ ሚያደርጉሰዎችብቻ ናቸውመረ ጃውን ሊያገ ኙየ ሚችሉት፡፡ይህ መረ ጃበሚጻፍበትጊዜየ እርሶ ሆነ ሌላሰውመረ ጃሊያይአይ ችልም፡፡

ጥያቄካለዎትሊጠይቁትየ ሚፈልጉትሰው

ምን ምጊዜ ጥያቄካሎትመጠየ ቅይችላሉ፡፡ተጨማሪ ጥያቄስለ ጥና ቱካሎትከዚህበታችየ ተገ ለፀ ውፁ ሰ ውማግኘትይችላሉ፡፡

ዳዉድጋሹ(ዶክተር)

አዲስአበባዩኒቨርስቲየ ምግብሳይን ስናኒ ውትሬሽን ማዕከል

በ ጥና ቱ ለ መሳ ተፍፈ ቃደኛ ከ ሆኑ ከ ዚህ በ ታች ባ ለ ው ቦ ታላ ይ ይ ፈ ር ሙ

ስ ለ ተ ሳ ትፎ አ መሰ ግ ና ለ ሁ፡ ፡

ስ ለ ጥና ቱ በ ደ ን ብ ተ ብ ራ ር ቶ ል ኛ ል እ ን ዲ ሁ ም ያ ለ ኝ ጥ ያ ቄ በ ደ ን ብ ተ መል ሶ ል ኛ ል ፡ ፡

በ ዚ ህ ጥ ና ት ለ መሳ ተ ፍ ተ ስ ማም ቻለ ሁ፡ ፡

………………… ……………….. ………………

ፊ ር ማስ ምቀ ን

………………………… ……………………………… ……………………

የ ዋ ና ው አ ጥ ኚ ፊ ር ማስ ምቀ ን

Annex10.5 Logistic Regression output Table

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	DD_Cat			3.355	2	.187			
	DD_Cat(1)	.955	.528	3.274	1	.070	2.598	.924	7.305
	DD_Cat(2)	.196	.244	.646	1	.422	1.216	.754	1.961
	Animal_Source_Food(1)	.747	.246	9.202	1	.002	2.111	1.303	3.420
	Hgn_Cat(1)	.815	.498	2.676	1	.102	2.259	.851	5.998
	morbdity(1)	.684	.236	8.430	1	.004	1.982	1.249	3.144
	FFQ_Coffee_24hr(1)	.778	.231	11.368	1	.001	2.178	1.385	3.423
	CRP_Cat(1)	.909	.273	11.098	1	.001	2.483	1.454	4.239
	Trimester			1.862	2	.394			
	Trimester(1)	.601	.515	1.360	1	.244	1.824	.664	5.009
	Trimester(2)	.308	.326	.893	1	.345	1.361	.718	2.577
	Constant	-2.298	.595	14.899	1	.000	.100		

a. Variable(s) entered on step 1: DD, Animal_Source_Food, Hgn_Cat, morbdity, Coffee_intake, CRP, and Trimester.

Annex10.6 Consent Letter form University and Addis Ababa Health Office

COLLEGE OF NATURAL SCIENCES
Addis Ababa University

የተፈጥሮ ሣይንስ ኮሌጅ
አዲስ አበባ ዩንቨርሲቲ

OFFICE OF THE DEAN
የዲን ጽ/ቤት

Ref.
ቁጥር CNSDO/225/16

Date.
ቀን February 4, 2016

To Whom It may Concern

College of Natural Science Institutional Review Board (CNS-IRB) in its meeting held on December 31, 2015 has reviewed an MSc thesis project proposal entitled **"Prevalence of zinc deficiency, and its associated risk factors among pregnant women from selected health centers in Addis Ababa, Ethiopia."** by Kebebew Regassa from Center for Food Science and Nutrition.

The proposal was approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean, College of Natural Science

ስልክ/Tel: +251-11-123 94 72
ፋክስ/Fax: +251- 11- 123 94 69
ፖ.ሣ.ቁ/P.O.Box: 1176Addis Ababa, Ethiopia
ኢሜል/Email: dean_cns@aau.edu.et

Please quote our reference number in your correspondence.

"Exmine all things; hold fast that which is good"

"ሁሉን መርምሩ መልካሙን ያዙ"

Reference AAHB/3104/227
Date 10/12/2015

To KOLFE HEALTH CENETR
MICKYLILAND HEALTH CENETER
SELAM HEALTH CENETR
SHEGOLE HEALTH CENTER

Addis Ababa

Subject: Request to access Health Facilities to conduct approved research

This letter is to support **KEBEBEW REGASSA to** conduct research, which is entitled as "**PREVALENCE OF ZINC DEFICIENCY AND ITS ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN FROM SELECTED HEALTH CENETRS IN ADDIS ABABA,ETHIOPIA** ". The study proposal was duly reviewed and approved by Addis Ababa Health Bureau IRB, and the principal investigator is informed with a copy of this letter to report any changes in the study procedures and submit an activity progress report to the Ethical Committee as required.

Therefore we request the Health facility and staffs to provide support to the Principal investigator.

With Regards

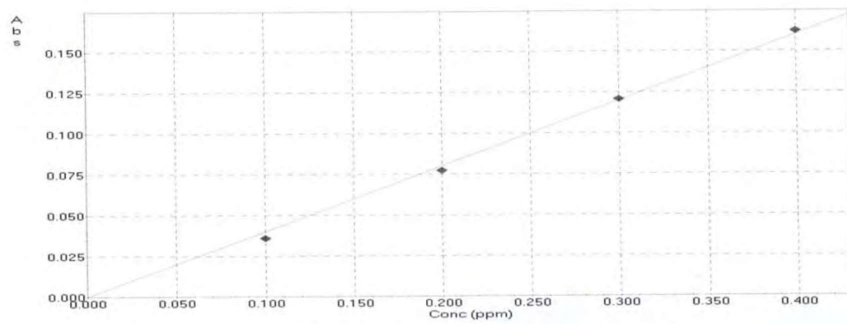
Eyobed Kaleb
Ethical Clearance committee

Cc KEBEBEW REGASSA
Addis Ababa
To Ethical Clearance Committee
Addis Ababa

Annex 10.7. Standard Calibration Curve for Serum Zinc level Determination

Saturday, October 01, 2016

Calibration Curve(Element:Zn:FlameCont C#:01)



Abs=0.40020Conc+0.00000 r=0.9999

CONC	ABS
0.1000	0.0359
0.2000	0.0771
0.3000	0.1207
0.4000	0.1621