ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

FOOD SCIENCE AND NUTRITION PROGRAM

Prevalence and Risk factors of Zinc status among Infants and Preschool children: a croos-sectional study in East Gojjam, Amhara region, Ethiopia

By Adamu Belay

A Thesis Presented to the school of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

Approved By Examining Board:

Dr. Melaku Umeta (Examiner) ____________

Prof. Negussie Retta (Examiner) ____________

Dr. Gulelat Desse (Advisor) ____________

Dr. Grace S.Marquis (Advisor) ____________

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

FOOD SCIENCE AND NUTRITION PROGRAM

Prevalence and Risk factors of Zinc status among Infants and Preschool children: a croos-sectional study in East Gojjam, Amhara region, Ethiopia

By Adamu Belay

A Thesis Presented to the school of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

Approved By Examining Board:
Dr. Melaku Umeta (Examiner) __________
Prof. Negussie Retta (Examiner) __________
Dr. Gulelat Desse (Advisor) __________
Dr. Grace S.Marquis (Advisor) __________

Acronyms

AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
EDHS	Ethiopian Demographic and Health Survey
EHNRI	Ethiopian Health and Nutrition Research Institute
ENA	Emergency Nutrition Assessment
FANTA	Food and Nutrition Technical Assistance
FAO	Food and Agriculture Organization
FFQ	Food Frequency Questionnaire
FMHACA	Food, Medicine and HealthCare Administration and Control Authority
HDDS	Household Dietary Diversity Score
HFIAS	Household Food Insecurity Access Scale
IZiNCG	International Zinc Nutrition Consultative Group
NNBSR	National Nutrition Baseline Survey Report
PCA	Principal Component Analysis
SPSS	Statistical Package for Social Science
UNICEF	United Nations Children's Fund
UND	United Nation University
WHO	World Health Organization

Acknowledgement

First and foremost, I would like to thank God for the good health and strength given to me to accomplish this work.

I would like to express my deepest gratitude and thanks to my advisors, Dr. Grace S. Marquis and Dr. Gulelat Desse, who have extended their heartfelt advice and suggestions on the preparation of the proposal and write up of this study. I highly appreciate their friendly and endless encouragement and guidance until the completion of this work.

I would like to extend my heartfelt thanks to Dr. Samson Gebremedhin who has guided me in the statistical analysis. I also thank Ato Dawd Gashu who has been participated at field for data collection and working as coordinator. I also thank all study subjects who were participated in this study. I also thank Meseret, Girma and Aregash Samuel for their kind technical, moral and material support.

I also thank Bikilla and Bekele for facilitating AAS instruments and other accessories in FMHACA laboratory.

I would like to thank the center for Food Science and Nutrition of Addis Ababa University for all rounded assistance and the School of Graduate Studies of Addis Ababa University who deserve special thanks for partially financing this work. I also would like to express my heartfelt thanks to Micronutrient initiative for partially funding this work.

Finally, I thank EHNRI for sponsoring me to join the School of Graduate Studies of Addis Ababa University and for allowing me to carry out the research in the laboratory of the institute.

Abstract

Zinc deficiency is a major public health concern and one of the most prevalent micronutrient deficiencies in developing countries, including Ethiopia. There was no information on serum zinc status of infants and preschool children in Ethiopian. The main objective of this study was to assess the prevalence and risk factors of zinc deficiency among infants and preschool children. A community based, cross-sectional study was conducted in East Gojjam zone between October and April 2011. Randomly selected 240 infants and preschool children were included in the study. Data on potential determinants of zinc deficiency were collected using a structured questionnaire. Serum zinc concentration was measured using Atomic Absorption Spectrometer. Anthropometric measurement analysis was done by using Emergency Nutrition Assessment 2011 software. Statistical analysis was done using ANOVA and Student's t-independent test and linear regression model. The mean serum zinc concentration of infants and preschool children was 62.98 (±13.03) μg/dl (95% CI: 61.32, 64.63 μg/dl). About 57.1% of the subjects were zinc deficient. Height-for-age, weight-for-age and weight-for-height revealed that 43.3% (95% CI: 37.10, 49.60), 19.7% (95% CI: 15.20, 25.30) and 5.9% (95% CI: 3.60, 9.70) of the total subjects were found to be stunted, underweight and wasted, respectively. The main determinants of low serum zinc status of infants and preschool children were age and number of family members living on the same land. Compared to infant age groups, zinc status of older children is 3.67μg/dl (95% CI: -5.58, -1.77 μg/dl) lower than children who were aged 6-10 months. Serum zinc status of infants and preschool children is decreased by 0.83 μg/dl (95% CI:-1.36, -0.30 μg/dl) with one unit increase the number of family members. Household food insecurity level, dietary diversity, sex, child health, anthropometric indices, maternal education, and socioeconomic status were not associated with serum zinc status. The prevalence of zinc deficiency was more than two-fold of the value set by IZiNCG. Such potential deficiencies require urgent attention needs including complementary food preparation education; traditional phytate reduction method and family planning implementation are recommended in the study area.

KEYWORDS: Serum zinc concentration, Zinc deficiency, Infant and preschool children

1- Introduction

1.1. Background

Micronutrient deficiencies such as vitamin A, iron, zinc and iodine are important nutritional problems and are widespread in many developing countries, including Ethiopia, and remain as the major problems among preschool children. The consequences of micronutrient deficiencies include psychomotor development retardation, impaired cognitive function, and growth retardation. The etiology of multiple micronutrient deficiencies is multifactorial; inadequate intakes, genetics, and infectious diseases may all play a role. In developing countries, poor dietary quality is often a major determinant of inadequate micronutrient intakes due to the diets based on plant based, and often contain very small amounts of animal source foods, and socio-demographic factors also affect zinc status of infants and preschool children. Trace elements are essential nutrients with regulatory, immunologic, and antioxidant functions resulting from their action as essential components or cofactors of enzymes in metabolism. More than 25% of enzymes in the human body need to be activated by metal ions to carry out their metabolic functions.

The importance of zinc is reflected by the numerous functions and activities over which it exerts a regulatory role. Zinc is also needed for nucleic acid metabolism and protein synthesis, cellular differentiation and replication, as well as glucose metabolism and insulin secretion since; zinc is required for the structure and activity of more than 200 enzymes.

Globally, about 2 billion people are zinc deficient. In 2002 World Health Report, zinc deficiency was included as a major risk factor to the global and regional burden of disease, along with iron, vitamin A and iodine deficiencies and to tackle the problem supplementation and food fortification programs were recommended. Zinc interventions are among those proposed to help reduce child deaths globally by 63%. Populations in South East Asia and sub-Saharan Africa are at greatest risk of zinc deficiency. Zinc intakes are inadequate for about one-third of the population and stunting affects 40% of preschool children. Zinc is commonly the most deficient nutrient in complementary food mixtures fed to infants during weaning. To date, the extent of zinc deficiency among infants and preschool children remains unknown whereas there were no previous studies conducted at the country and regional level including Amhara region. Therefore, the aim of this study was to determine the prevalence and risk factors of zinc deficiency among infants and preschool children in East Gojjam, Amhara region of Ethiopia.

1.2. Statement of the problem

Malnutrition remains a common health problem among children under 5 years worldwide. In the past, malnutrition was thought to be primarily due to insufficient intake of macronutrients; however, micronutrients seemed to play also an important role. During the past two decades, deficiencies of three micronutrients have received the attention globally, namely the iodine, vitamin A and iron (FAO/WHO, 2002). Zinc deficiency is wide spread in low-income countries and is responsible for 4% of childhood deaths and 1% of the burden of disease in Africa, Latin America and Asia (Fischer et al., 2009). About one third of the populations in sub-Saharan Africa and South East Asia have the greatest risk of zinc deficiency because of inadequate zinc intake (Brown, et al., 2004). In Ethiopia Umeta et al., (2000) conducted an experimental study in infants at Arsi district, and the finding shows that there was zinc deficiency problem in Ethiopia. Supplements of zinc as micronutrient may be very important, because zinc deficiency is associated with stunting and poor growth (Brown et al., 2002). In our country there is no database about zinc deficiency infants and preschool children. To my knowledge, in our country the zinc deficiency is estimated by stunting rate of children, also the information about the prevalence of zinc deficiency in our country estimated by indirect analysis based on the countries food balance sheet (IZiNCG, 2004). According to national nutrition survey conducted in 2009, it was noticed that several children were stunted. On the other side, the extent of zinc deficiency among infants and preschool children remains unknown whereas there are no previous studies conducted. There is an urgent need to have information for database, in order to find out the status of zinc, stunting and to study the presence of some other risk factors for zinc deficiency. The study results can help policy makers to develop strategies to overcome the problem.

1.3. Significance of the study

Assessing the zinc status of a population is critical in developing nutrition intervention programs that enhance human health and well-being. The results of zinc nutrition assessment efforts are necessary both to determine the presence and magnitude of zinc deficiency problems and, when indicated by the results, to elicit public interest and collect information for appropriate action. Assessment data are used to determine the level of risk of deficiency and indicate the risk factors which contribute to low zinc status in the general population. Information derived from assessments is also used to identify specific segments of the population at elevated risk so that interventions may be targeted to those in greatest need, or to determine whether population-wide interventions are indicated. Assessments can also be used to monitor changes in zinc status of subjects after intervene over time, thereby permitting decisions on the effectiveness of intervention programs and need for their continuation.

Community-based data relating to prevalence of zinc deficiency and factors influencing zinc deficiency among preschool children in Ethiopia are scarce. Determining the magnitude of zinc deficiency and its association with risk factors among preschool children in Ethiopia can contribute to planning strategies to alleviate zinc deficiency and the related adverse health consequences. Therefore, the study will provide valuable information to the Amhara regional state and policy makers regarding the magnitude of the deficiency of zinc among preschool children of East Gojjam. This will be useful for development of policy and intervention to tackle low zinc status in the community.

1.4. Objectives of the study

1.4.1. General objective

- The overall objective of the study was to assess the prevalence and risk factors of zinc deficiency among infants and preschool children in East Gojjam.

1.4.2. Specific objective

- To determine the prevalence of zinc deficiency in infants and preschool children in East Gojjam.
- To identify the risk factors of zinc deficiency among infants and preschool children in East Gojjam.

2. Literature review

2.1. Function of zinc

Zinc is the second most common trace element in the body after iron. Zinc is involved in the functioning of nearly 200 different enzymes. These enzymes help protect cells from free radical damage and are needed for the synthesis of DNA and RNA, carbohydrate metabolism, acid-base balance, and folate absorption. Zinc plays a role in the storage and release of insulin, the mobilization of vitamin A from the liver, and the stabilization of cell membranes. Zinc is important in gene expression and therefore is needed for the growth and repair of tissues, the activity of the immune system, and the development of sex organs and bone. Zinc-containing proteins are needed for the activity of vitamin A, vitamin D, and a number of hormones including thyroid hormones, estrogen, and testosterone. Without zinc, these nutrients and hormones cannot bind to DNA to increase or decrease gene expression and, hence, the synthesis of certain proteins (Smolin and Grosnenor, 2011).

2.2. Major sources of zinc

In the diet, zinc is found in red meat, liver, eggs, dairy products, vegetables, and some seafood. Whole grains are a good source, but refined grains are not because the zinc in these grains is lost in milling and not added back in enrichment. Zinc is better absorbed when it is ingested from animal products than it is from plant sources because the zinc in plants is often bound to Phytate, fiber, oxalates, and tannins. Grain products leavened with yeast or naturally fermented provide more zinc than unleavened products because the yeast leavening reduces the phytate content (Smolin and Grosnenor, 2011). Also, lactic acid fermentation of cereals is a long established processing method to degrade phytate from cereal products as a result bioavailability of zinc is increase (Haros et al., 2008).

2.3. Micronutrient deficiency

Micronutrient deficiencies are common in many developing countries and are typically due to inadequate food intake, poor dietary quality, poor bioavailability (because of the presence of inhibitors, mode of preparation, and interactions), and/or the presence of infections. Micronutrient status can affect health outcomes such as child survival, growth, and development, either directly (e.g., deficiencies of vitamin A, iodine, iron, zinc, or folic acid) or indirectly through interactions with each other (e.g., interactions with vitamin A, zinc, or iron), increasing food intake owing to improved appetite, and reducing morbidity. Most discussions of micronutrient deficiency have been limited to the "Big 3," namely vitamin A deficiency (VAD), iodine deficiency disorders (IDD), and iron deficiency anemia (IDA). While, these problems remain significant public health concerns, it is important to recognize other micronutrient deficiencies such as zinc, folate, and multiple micronutrient malnutrition (Ramakrishnan, 2002).

Deficiencies of micronutrients are a major global health problem. More than two billion people in the world today are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc. Most of these people live in low income countries and are typically deficient in more than one micronutrient. Deficiencies occur when people do not have access to micronutrient-rich foods such as fruit, vegetables, animal products and fortified foods, usually because they are too expensive to buy or are locally unavailable. Micronutrient deficiencies increase the general risk of infectious illness and of dying from diarrhea, measles, malaria and pneumonia. These conditions are among the ten leading causes of disease in the world today (WHO, 2001).

The groups most vulnerable to micronutrient deficiencies are pregnant women, lactating women and young children, mainly because they have a relatively greater need for vitamins and minerals and are more susceptible to the harmful consequences of deficiencies. For a pregnant woman, these include a greater risk of dying during childbirth, or of giving birth to an underweight or mentally-impaired baby. For a lactating mother, her micronutrient status determines the health and development of her breast-fed infant, especially during the first 6 months of life. For a young child, micronutrient deficiencies increase the risk of dying due to infectious disease and contribute to impaired physical and mental development.

2.3.1. Zinc deficiency

Zinc deficiency was first documented in malnourished adolescents in the Middle East in 1960s. The deficiency was characterized by growth retardation, hypogonadism and delayed sexual maturation. One of the first symptoms of zinc deficiency is poor appetite and changes in the perception of taste and smell. Lethargy, slowing of activity, apathy and depression are also associated with zinc deficiency. Impaired cell-mediated immunity, slow wound healing, dermatitis and failures to thrive are other manifestations of this nutritional problem. Zinc is prevalent in the brain. There is evidence to indicate that zinc deficiency in infants may lead to deficits in children's neuropsychological functions, activity or motor development subsequently interfering with their cognitive development and performance (King, 1990).

The etiology of zinc deficiency may be dietary or physiologic. Dietary deficiency can be due to lack of zinc in foods consumed. It may also be caused by the presence of substances, described earlier, that interfere with zinc absorption. Various disorders of the gastrointestinal tract may result in zinc depletion; for example, severe intestinal malabsorption syndrome, enter colitis, short bowel syndrome, celiac disease and cystic fibrosis. Plasma zinc levels may be depressed in infectious hepatitis, alcoholism, protein-energy malnutrition, and sickle cell anemia. Acrodermatitis enteropathica is a rare genetic disease characterized by alopecia, dermatitis, diarrhea, photophobia, psychological changes, failure to thrive, infections and death. The disease is believed to be due to a defect in zinc absorption, most likely due to insufficient synthesis of the low molecular weight zinc ligand. Zinc deficiency in breast-fed only children is a rare disorder affecting mostly premature infants. However, some cases of the disease in term infants have also been reported. Acrodermatitis enteropathica first manifests itself upon weaning from breast milk. The disease may be treated by continuing breastfeeding or through supplemental zinc (Yeung and Laquatra, 2003).

Deficiencies of zinc have been reported in populations that consume diets based on cereal proteins from which zinc is poorly absorbed. Symptoms include poor growth and development, skin rashes, impaired reproduction, and decreased immune function. It can affect the number and function of immune cells in the blood and therefore can lead to an increased incidence of infections. High intakes of zinc can also impair immune function, as well as increase the risk of heart disease and interfere with copper absorption. Zinc is often marketed as a supplement to improve immune function, enhance fertility and sexual performance, and cure the common cold. Supplements have been shown to reduce the incidence of diarrhea and infections in people who have low zinc levels, but there is no evidence that extra zinc is beneficial. Over-supplementation may result in toxicity (Smolin and Grosnenor, 2011).

Diarrhea is a condition that has a major impact on global health, is highly correlated with nutritional status. It is an important area of focus not only due to its high worldwide prevalence and health costs, but because it can be significantly reduced by appropriate interventions and treatment. Diarrhea is a major killer disease in children under five years of age, and recurrent diarrhea affects nearly 20% of the population, thus it is an important public health problem (WHO, 1998). Infectious diarrhea also contributes to malnutrition due to a decreased nutritional intake and diminished absorption of vital nutrients during the acute episode and recovery period. Malnutrition, in turn, decreases the ability of the immune system to fight further infections, making diarrheal episodes more frequent. Given its involvement in numerous biologic functions, health depends upon normal levels of zinc, particularly immunity. It is not surprising to find that zinc deficiency is associated with numerous infectious illnesses. Diarrhea leads to loss of zinc and abnormalities of zinc metabolism. Substantial amounts of zinc are lost during acute diarrhea: daily losses of zinc during diarrhea can be as high as 160μg/kg per day in children (Castillo et al., 1988).

It is now believed that zinc deficiency plays a major role in stunting. The prevalence of zinc deficiency is unknown definitely in developing countries, but mild and moderate forms are likely to be wide spread by using food balance sheet and stunting rate. A study conducted by Umeta et al., (2000), showed the presence of zinc deficiency in Ethiopia. In their intervention study, it was found that combating zinc deficiency can increase the growth rate of stunted children to that of non-stunted children in rural Ethiopia and called for the need for zinc supplementation. Besides halting the stunting process in infants, zinc supplementation would be essential for catch up growth, improving general health status, stimulating appetite and reducing morbidity due to several relating to infants' diseases.

Dietary sources of zinc are protein rich foods including meat, fish and shellfish and whole grains. Root and tuber crops are low in their zinc content, while zinc in cereal staples is most often poorly bioavailable. Where diets are plant based and intakes of animal foods are low, the risk of inadequate intake of zinc is very high even when energy and protein intakes meet recommended levels. In these circumstances bioavailability rather than amount is the critical factor. In light of this fact, in most of the rural areas of Ethiopia, consumption of animal sources is mostly limited to occasional public holidays, which indicates minimal intake. Thus one can expect a high prevalence of zinc deficiency in Ethiopia. It might also be the reason for the observed high prevalence of stunting in this country (Getahun et al., 2001). In early study of Umeta et al., (2000) highlights the presence of zinc deficiency in infants who lived at Arsi district. Recent population level analyses from food balance sheets have estimated that 21% ranging from 3% to 73% of the world's population was at risk from zinc deficiency. Children especially in developing countries are suffering from zinc deficiency due to chronic inadequate intake of zinc rich food (Brown et al., 2004) and IZiNCG, 2004 estimated that about 21% of Ethiopian populations are at risk of zinc deficiency. Also, recent studies on zinc deficiency in pregnant women in Sidama, Southern Nation by Gibson et al., (2008) and Gebremedhin et al., (2011) revealed that there were a high prevalence of zinc deficiency in the study area, but there was no data regarding zinc status in infants and preschool children.

2.4. General methods of assessing risk of zinc deficiency

Poor zinc nurture and possible zinc deficiency is likely to be widespread in most developing countries (Gibson, 1994 and Sandstead, 1991), but the quantitative estimate of the global prevalence is not known because of the difficulty in assessing zinc status. However, it may be estimated indirectly using proxy information on zinc intake from global food supply (Brown et al., 2001) and secondly by reviewing the national prevalence of stunting in children (De Onis et al., 2000).

The diets of developing countries are largely based on cereals and legumes staples and based on the phytate: zinc molar ratio exceeds >15, the bioavailability of zinc is estimate at 10-20%. Using this approach, the amount of zinc present in the foods that is potentially absorbable has been calculated and the adequacy of food supply to satisfy the population's theoretical requirements for zinc has been assessed. Based on these assumptions, current estimates indicated that about 95% of South Asian, 74% North African and Mediterranean and 68% of sub-Saharan African populations are at risk of low dietary intake of zinc (Brown et al., 2001). On average, about 48% of the world populations are at a risk of zinc deficiency.

The global database also provides information on national prevalence of childhood stunting (De Onis et al., 2000). One of the earliest signs of suboptimal zinc nurture in infant and children is impaired growth. Those countries with high prevalence of stunting tend to be those with high risk of low zinc intakes. These relationships may suggest that high prevalence of stunting may be used as a proxy indicator for zinc deficiency in the absence of a reliable biomarker.

As with other nutrients, a number of general techniques can be used to estimate the risk of zinc deficiency in individuals or in populations. These are categorized as the following:

Method 1: The presence or prevalence of clinical outcomes of zinc deficiency (e.g., stunting, diarrhea), or ecologic factors associated with risk of zinc deficiency or risk of inadequate zinc intakes;

Method 2: Measurement of functional responses following the intake of adequate supplemental zinc. (UNU, 2004)

Method 3: Assessment of the adequacy of dietary zinc intakes in relation to theoretical requirements for absorbed zinc;

Method 4: Biochemical measures of zinc concentration, activity of zinc-dependent enzymes, or other zinc responsive biocomponents in biologic fluids or tissues, assessed in comparison to reference values or established cutoffs.

Method 1: Prevalence of stunting

For the first method, the presence or prevalence of clinical outcomes of zinc deficiency (e.g., stunting, diarrhea), has been well established, both by studies in experimental animals and by human intervention trials, that zinc deficiency is growth-limiting. In a recent meta-analysis, the results of more than thirty community-based intervention trials completed in different parts of the world were examined to determine the overall magnitude of growth responses to zinc supplementation (Brown et al., 2002). Notably, the responses to zinc supplementation were significantly greater in those studies that enrolled subjects with preexisting nutritional stunting or underweight, defined respectively as height-for-age or weight-for-age Z-scores < -2 in relation to international reference data. By contrast, there were no significant effects of zinc supplementation in those studies that enrolled mostly children who were non-stunted and/or had adequate weight-for-age. These results indicate that children with low height-for-age or weight-for-age are likely to be zinc deficient, and they further suggest that the national prevalence of stunting or underweight among children under 5 years of age can be used as indirect indicators of a population's risk of zinc deficiency.

The aforementioned meta-analysis found no effect of zinc supplementation on weight-for-height indices, suggesting that zinc mostly affects linear growth. Thus, the rate of stunting, or low height-for-age, is probably the best anthropometric indicator of risk of zinc deficiency. The WHO considers national stunting rates of ≥ 20% to be a level of public health concern (WHO, 1995). The same cutoff can be applied to indicate when there may be a substantial risk of zinc deficiency, in which case further assessment of zinc status should be considered. Data on the prevalence of stunting are collected routinely in many countries and are compiled in the WHO

Global Data Base on Child Growth and Malnutrition (WHO, 1997). This method is the indirect method of assessing the risk of zinc deficiency in the population.

Method 2: Functional indicators: response to supplementation

Because of uncertainties in the interpretation of the foregoing techniques to assess zinc status, many recent studies have relied on the identification of a functional response to zinc supplementation as the basis for diagnosing preexisting zinc deficiency in the supplemented individuals. Use of a functional response to indicate deficiency requires randomly administering either zinc or placebo to members of the target population and comparing the responses in the two groups of subjects. Ideally, the subjects are selected to be a representative sample of a larger population, so inferences can be drawn for the population as a whole. Functional responses that have been used previously include physical growth, immune function and rates of specific infections, physical activity and performance on psychometric tests, and hormonal responses, among others. Retardation of physical growth is an early and prominent feature of experimental zinc deficiency in young animal. There are also indications that maximal growth potential can be limited by zinc deficiency in infants, children and adolescents. The growth of male infants fed a zinc supplemented formula was greater than that of control infants fed the same formula without a zinc supplement. The major disadvantage of using these functional responses to zinc supplementation as indicators of zinc deficiency is the long delay that is often required for the response to be detectable and the consequently high cost of this diagnostic technique. Appropriate efforts to ensure that the supplements are actually consumed contribute further to the cost of these assessments (Walravens et al., 1983).

Method 3: Dietary intake method of zinc assessment

Zinc deficiency is expected to be widespread. However, to move forward with the development and evaluation of programs to improve population zinc status, it is necessary to derive more precise estimates of the magnitude of risk of zinc deficiency using more direct measures of a population's zinc status. Currently, national prevalence estimates of zinc deficiency based on direct measures are lacking for most countries. The methods for assessing the risk of population zinc deficiency include assessment of dietary intakes of zinc, biochemical indicators of zinc status, and functional response to zinc supplementation. These suggest methods for the direct measurement of zinc status, and possible approaches to the interpretation of results; application of standardized methods will assist in the comparison of information on indicators of zinc status of different populations (Gibson et al., 2008).

The assessment of dietary intakes of zinc in the population is important because inadequate dietary intake of zinc is the most likely cause of zinc deficiency; dietary assessment is an important component in evaluating the risk of zinc deficiency. Information on the adequacy of dietary zinc intakes should be interpreted together with data derived from other assessment methods, such as biochemical assessment, to facilitate interpretation of the risk of zinc deficiency in the population. Standard dietary assessment methods can be applied to evaluate the adequacy of zinc intakes in populations, and to support results of biochemical assessments. Further, information derived from dietary surveys is useful for determining the specific dietary causes of inadequate zinc intake and therefore to help identify appropriate food-based approaches to intervention (Gibson and Ferguson, 1999).

There are quantitative methods for assessing usual dietary intakes of individuals: weighed food records, recalls, and semi-quantitative food frequency questionnaires (Gibson, 1990; Cameron and Staveren, 1988). Of these, food records and recalls are designed to measure the quantity of each food consumed over a one-day period. By contrast, a 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.

Weighed food records completed by trained research assistants in households have been used to collect reliable quantitative data on dietary intakes, including zinc, in lower-income countries (Ferguson, et al., 1994). This method is more time consuming and costly, it has a higher respondent burden than other methods, and may increase the likelihood that respondents change their dietary intakes during the recording period; this may be leads to erroneous outcomes. However, weighed food records are the most accurate method of determining actual intakes during the recording period.

Dietary recalls can be used for estimating zinc intakes among non-literate populations, provided that portion sizes of the staple can be recalled accurately (Ferguson et al., 1995; Rutishauser, 1973). Proper training of field workers in recall interview techniques can minimize bias and non-response rates (Gibson, 1997). Several strategies as well can be used to reduce memory lapses and facilitate portion size estimates, including training respondents in the use of food picture charts, bowls, plates and utensils familiar to the locale, and samples of actual cooked or raw foods that are commonly consumed (Ferguson et al., 1995). Recalls are suitable for areas where diets are not very diverse and are predominantly plant based, as is the case in many lower-income countries. Although some accuracy is compromised by their use, recalls are easier, faster, and less expensive than weighed food records and are less invasive, so that compliance is enhanced, and the tendency to alter food intake is reduced. An interactive 24-hour recall method has been specially designed for measuring usual intakes of total and absorbable zinc in lower-income countries. The feasibility and the relative and concurrent validity of this method were tested in rural Malawi, in sub- Saharan Africa (Gibson and Huddle, 1998; Ferguson et al., 1995). Intakes of available zinc calculated from three interactive 24-hour recalls and indices of absorbable dietary zinc were significantly associated with hair zinc concentrations, confirming that this assessment method can provide valid estimates of the amount of zinc available for absorption at the individual level. Further validation of this method to estimate usual intakes of absorbable zinc by individuals in other populations would be useful. Details of this interactive 24-hour recall method for determining intakes and adequacy of absorbable and total zinc (and iron) are given in Gibson and Ferguson (Gibson and Ferguson, 1999).

Semi-quantitative FFQs have not yet been validated for the estimation of usual zinc intakes by individuals. Unlike nutrients such as vitamin A and calcium, which are concentrated in a relatively small number of foods or specified food groups, zinc occurs in a wide range of plant based food items as well as animal source foods and therefore may be quantified less accurately using this method. Although FFQs may prove to be suitable for determining mean population intakes, more research is required on the validity of this technique for estimating usual intakes of absorbable zinc for individuals before it can be applied with confidence (Hotz, 2007).

FFQ provide information about how often certain foods or foods from given food groups, were eaten during a time interval in the past, usually day, by either the household or an individual. The questionnaire can be self-administered or be administered through a short personal interview. The food list may range from a few questions to capture intake of selected foods and nutrients, to a comprehensive list to assess the total diet. The frequency responses can be open-ended or multiple choice, ranging from several times per day to number of times per year, depending on the type of food. FFQ can be qualitative with no information on portion size, semi-qualitative with standardized portion size estimates (as predetermined by the interview team), or quantitative where the respondents estimate portion size. When portion sizes are described by the respondents themselves, different measurement aids have been used, such as photographs, drawings or household measures. Portion size information is necessary to quantitatively assess the intake of foods and nutrients. Standard portion sizes greatly simplify the administration and processing of the FFQ.

Lacey, (2007), was conducted a research to estimate the adequacy of zinc intake among college students by using semi quantitative-FFQ, the finding of the research showed 76% of the women who were obtaining less than the National Academy of Sciences Recommended Dietary Allowance for zinc (8 mg/ day for females, 11 mg/ day for males).

Serra-Majem et al., (2009) were conducted to validate dietary assessment methods for intakes of iron, calcium, selenium, zinc and iodine by comparing 24-hour recall and dietary record methods for the European Community's 'EURopean micronutrient RECommendations Aligned' (EURRECA) Network of Excellence, whose aim is to harmonize nutrient recommendations across Europe. The FFQ was seen as a valid method for assessing mineral intake, particularly for Ca and, to a lower extent, for iodine and Zn. Se and Fe showed only acceptable correlations. This finding provides new insights regarding the characteristics that assessment method for dietary mineral intakes should fulfill. So, the advantages include simpler and quicker administration and processing, and subsequently lower costs as well as fewer burdens for the respondents than alternative methods. The method is generally accepted as being suitable for measuring typical diets and with the purpose of ranking individuals according to intake.

Inaccuracies may result from an inadequate listing of possible foods, errors in estimating portion size and the usual frequency of food consumption.

Method 4: Serum zinc concentration

The fourth direct method to assess the risk of zinc status in population is serum and plasma zinc concentrations which are the most widely used biochemical markers of zinc status. Strictly speaking, zinc concentrations measured in serum or plasma is not entirely comparable, largely as a consequence of the different collection and separation procedures used. Circulating zinc concentrations may have limitations in validity and reliability regarding the identification of mild to moderate zinc deficiency in individuals. Several lines of evidence suggest that this index is useful in assessing zinc status at the population level (IZiNCG, 2007; Brown et al., 2002; Brown, 1998 and King, 1990). In particular, the mean serum zinc concentration of groups of individuals responds as expected to dietary modifications and in association with functional outcomes following zinc supplementation. For example, in the United Kingdom National Diet and Nutrition Survey, there was a significant positive correlation between dietary zinc intakes, assessed by seven-day weighed food records, and fasting serum zinc concentration for girls aged 4 to 18 years, although not for boys (Gregory et al., 2000). In a study of New Zealand women not using oral contraceptives, there were significant negative correlations between serum zinc concentrations and intakes of dietary phytate and the phytate: zinc molar ratio (Gibson et al., 2001). An inverse correlation between serum zinc and dietary phytate: zinc molar ratios have also been reported in adolescent Canadian women consuming lacto-ovo vegetarian, semi-vegetarian, and omnivorous diets (Donovan and Gibson, 1995). Further, in two experimentally controlled studies in which healthy adults were first fed omnivorous diets and then switched to vegetarian diets, measurable declines in serum zinc values on the vegetarian diets were reported (Hunt et al., 1998; Srikumar et al., 1992).

In an earlier meta-analysis of the effect of zinc supplementation on children's growth, which included studies of severely malnourished in-patients, the initial mean serum zinc concentration was inversely associated with the magnitude of the growth response to zinc supplementation (Brown et al., 1998). However, this relationship was not statistically significant in an updated version of the meta-analysis that excluded severely malnourished children (Brown et al., 2002). Low serum zinc concentration was found to be predictive of increased risk of diarrheal morbidity among Indian children (Bahl et al., 1998). In the aforementioned meta-analysis of the effect of zinc supplementation on children's growth, data on mean serum zinc concentration before and after the intervention were available for 15 studies (Brown et al., 2002). As evidence, the serum zinc concentration is better methods to assess zinc deficiency in a population due to biochemical indicators are an objective and quantitative means of assessing the zinc status of a population. They are useful for identifying populations and specific subgroups that are at elevated risk of zinc deficiency and therefore can be used to target interventions to specific high-risk groups. For a variety of reasons described below, Serum or plasma zinc concentration is the best available biomarker of the risk of zinc deficiency in populations. WHO, UNICEF, IAEA, and IZiNCG jointly recommended the use of serum zinc concentration for assessment of population zinc status (WHO/UNICEF/IAEA/IZiNCG, 2007).

For the correct use of serum zinc concentration as an indicator of zinc status, there are several important technical issues that must be considered regarding sample collection, laboratory analysis, and interpretation of the data. Why use serum zinc concentration as an indicator of zinc status?

The chosen serum zinc concentration has some important characteristics that make it a good indicator of zinc status for populations by three main reasons: it reflects dietary zinc intake, it responds consistently to zinc supplementation; and reference data are available for most age and sex groups.

To date, serum zinc is the only biochemical indicator of zinc status known to meet these criteria. Also, experimental studies of dietary zinc restriction in adult volunteers have found that the serum zinc concentrations of previously well-nourished individuals decline within a few days or weeks after their zinc intake is severely restricted. Some, but not all, studies of moderately restricted zinc intakes have shown that serum zinc declines, although the response takes longer and is less consistent. Research also shows that serum zinc concentration consistently rises when individuals consume zinc supplements in addition to their usual diet, regardless of their initial serum zinc concentration (Hess et al., 2007). Thus, there is strong

evidence to indicate that, in general, serum zinc concentration reflects a person's usual zinc intake during the previous few weeks or months. However, other factors can independently affect the serum zinc concentration. For example, infection can lower serum zinc concentration, while muscle break down during weight loss can liberate zinc to the circulation and increase serum zinc concentration (IZiNCG, 2007).
Thus, the population means serum zinc concentration is a useful indicator of successful delivery and absorption of zinc supplements in children. In addition several studies were done in the world to assess the micronutrients deficiency among the risk group of population by analyzing serum. In some studies conducted in Africa, among preschool children, 37.7% were severe zinc deficient and 9.8% were moderate zinc deficient in Nigeria (Bilbis et al., 2003) In a baseline study in Kenya, nearly all (95.7%) preschool children in Suba district were zinc deficient (Ohiokpehai et al., 2009). In Saudi Arabia, in Riyadh City, 18.3% of preschool children were moderately zinc deficient and 3.3 % were severely zinc deficient (Alshatwi, 2006).

Little information is available from nationally representative surveys on the prevalence of low serum zinc concentration; however, estimation of the extent of zinc deficiency must rely on the prevalence of stunting among children less than five years of age. As zinc deficiency is not the only factor affecting children's growth, assessment of serum zinc levels can be used to confirm the risk of zinc deficiency in countries having high prevalence of stunting. In Ethiopia, studies were conducted to assess the prevalence of zinc status in population is based on the stunting rate without biomarker (NNBSR, 2010). The present study will assess the prevalence of zinc deficiency among infants and preschool children by analyzing serum zinc, since serum and plasma zinc concentrations are the most widely used biochemical markers of zinc status (Gibson et al., 2008).

Among the above four general methods of assessing risk of zinc deficiency, serum zinc concentration is the best method due to three main reasons: it reflects dietary zinc intake, it responds consistently to zinc supplementation and a reference data are available for most age and sex groups.

3. Materials and methods

This study was conducted under the umbrella of the main project which being done in six zones of Amhara region namely, West Gojjam, East Gojjam, North Wello, South Wello, South Gondar and Wag Hemra entitled " *The effect of iodized salt on child development in Amhara region in Ethiopia*" (PI: Dr. Grace S. Marquis).

Study design: This is a cross-sectional quantitative study with descriptive and analytical elements.

3.1. Study area

The study was carried out between October 2011 and April 2012 including East Gojjam Zone, which is one of the administrative zone among eleven zones of Amhara region, located 150 km north-west of Addis Ababa, Ethiopia. In East Gojjam zone consists of 17 weredas: Among these weredas twelve weredas were included in this study namely, Hulet Ej enese, Goncha Siso enese, Enarj Enawga, Shebel Berenta, Dejen, Awabel, Aneded, Baso liben, Debay Tilatgin, Sinan, Bibugn and Gozamiin, the corresponding selected kebele from each woredas were Andinet mariyam, Buza Yemerat, Arigemit kenibata, Werobona akababiw, Wibilat Getem, Addisamba Chelia, Adisge yegewek, Yedug, Naziret, Washa Michael, Wenber Kidus Yohannes and Yebona Erjina, respectively. According to the 2007 Ethiopian census, this zone has a total population of 2,152,671, with male-to-female ratio of 0.98. With an area of 14,000 square kilometers, the zone has a population density of 153.80. The dominant crops in this zone include sorghum, teff, maize and haricot beans, grown for both consumption and commercial values. Only small fractions (9.92%) are urban inhabitants. This zone had a very high potential for irrigation, however, agriculture is totally rain-fed. Total annual rainfall ranges approximately from 900-1200 mm per year. The zone has a long term mean annual rainfall of 1181 mm, and in most years precipitation is very favorable for the cultivation of different crops. The main reason for selection of this zone is the infrastructure relatively better than the other zone and there was no information for East Gojjam on magnitude and distribution of zinc deficiency among infants and preschool children in this zone.

3.2. Study subjects

The source population is all children, aged below five years, categorized into three age groups: 6-10, 18-22 and 54-60 months. The aforementioned age groups were selected because the risk of zinc deficiency and stunting are greatest during the period of rapid body growth and development in these age groups, and one of the high risk group for zinc deficiency are infant and young children (WHO, 1995).

3.3. Sample size

Sample size was calculated using single proportion sample size calculation formula with inputs of 95% confidence level, (Z=1.96), 10% margin of error, 50% expected prevalence of zinc deficiency in order to get larger sample size and accordingly sub-sample size of 96 is calculated. From each age group 96 subjects were expected. So, total sample size of 288 was expected.

$n = Z_{\alpha/2} \times p\,q/d^2$

Allowable error = (10%), d = 0.1

$Z_{\alpha/2} = 1.96$ (at 95% CI)

Zinc deficiency rate of East Gojjam is not known, so, by considering 50% expected prevalence of zinc deficiency, p=0.50

$$q = 1-0.50 = 0.50$$

$$n = (1.96)^2 \times (0.5 \times 0.5) / (0.10)^2$$

$$= (3.8416) \times (0.25) / (0.01)$$

$$= 96$$

In the study three age groups were included, so, total sample size of 288 was expected.

3.4. Sampling technique

The main project has been used the 2007 census data as sampling frame and cluster as sampling method, the kebele has been cluster, include only rural Kebeles from the 6 zones namely; West Gojjam, East Gojjam, North Wello, South Wello, South Gondar and Wag Hemra.

Others zones in Amharas region were not included because (1) Amharic was not the main first language (e.g. Awi) or (2) close to University of Gonder field study areas (North Gonder) or (3) too closed to the salt production site, so may have early iodized salt (e.g. Oromia and North Shewa). Listed all the rural kebele which not take more than 3 hours walk from the point of accessibility of the car in six zones from 1 to the end and use a random number table to select the first kebele and so on till we got the 60 kebeles. Extra Kebeles then selected for replacement.

Before data collectors goes into the study area, the census team who were completed at least 10 grades were involved in this project and collected the name of head of household, mothers and their children by the help of extension workers, the extension worker role was to facilitate the things by assigning health workers in each sub-kebeles in the selected kebeles. The eligible child was selected by a random number table.

To determine the prevalence of zinc status approximately 240 mothers and their children under 60-months of age from 12 woredas in East Gojjam were randomly selected; from each woreda one rural kebele were selected in simple random method for the study. With the help of government-paid Health Extension Workers and the census team, from all selected kebeles of East Gojjam 90 children from 6-10 months, 70 children from 18-22 months and 80 children from 54-60 months were recruited. In the baseline study the census team listed all children from each age group in each sub-kebele by the help of Extension Workers and voluntary health workers. Eligible subjects were selected in a simple random method from the listed households in each kebele.

3.5. Data collection

Data have been collected by research assistants with bachelor and master's level degrees who have been trained on data collection. In the project, ten data collectors for dietary and socioeconomic information, ten for psychology, 4 individuals trained on anthropometry, and 4 phlebotomists/lab technicians participated. All enumerators were trained over ten days from April 2, 2011 to April 23, 2011 and then conducted field tests in order to assess inter-observer and test-retest reliability. With the help of Health Extension Workers, participants for the study identified by trained field workers.

Structured questionnaires for household, anthropometric measurement and serum zinc analysis were used as a tool for data collection. Mothers were interviewed at baseline concerning the family's socio-economic and demographic information, child's health and diet, and other related information (Annex 3).

3.6. Anthropometric measurement

Height of infants and preschool children aged 6-22 months was measured in a recumbent position to the nearest 0.1 cm according to the procedure outlined on anthropometric measurement guide using a board with an upright wooden base and a movable headpiece (WHO, 2006). Height of children 24 months and older was measured in a standing-up position to the nearest 0.1cm according to the procedure described anthropometric measurement guide.

Body weight of children was recorded to the nearest 0.1 kg by using Tanita scale. The weight of the children aged of 6-10 and 18-24 months, the mother was weighed with the child and then weighed without the child. The difference between the two measurements was the child's weight. Z-scores were calculated for the indicators: weight-for-age, height-for-age, and weight-for-height with ENA 2011 software using WHO (2006) reference population. Underweight, stunting, and wasting were defined as weight-for-age Z-scores <-2, height-for-age Z-scores <-2, and weight-for-height Z-scores <-2, respectively. Accuracy of all anthropometric measurements was upheld according to the international reference standard. Anthropometric data were measured twice if there were 0.05 SD between two measured values, the third measured were taken.

3.7. Blood collection

All precaution during blood sample collection for serum zinc analysis was considered according to IZiNCG, (2007) recommendation. The antecubital and infants head areas were cleaned with 70% alcohol and medical cotton. Five milliliters of venous blood was drawn using stainless steel butterfly needle, and collect into trace element-free evacuated blood collection tubes

(Vacutainers, 6 ml, and Royal blue top) without anticoagulant for processing serum, blood sample was placed in ice box and allowed to clot 30–40 minutes. Then, sera were separated from cells by centrifugation at 3000 rpm for 10 min at room temperature for forty minutes of collection and transported to the project office kept in cool boxes (0-4^{o}C). Sera were stored at –80^{o}C until serum zinc determination took place.

3.8. Determination of zinc

For the determination of trace elemental zinc, sera were shipped to EHNRI by dry ice and polystyrene packaging material. A concentration of trace elements in serum was determined at FMHACA laboratory by using Shimadzu Flame Atomic Absorption Spectroscopy (AA 6800 model, Japan). 200μL of serum sample were added into a trace metal free plastic test tube and diluted by addition of 6% butanol in 1:5 ratios. Calibration of the Atomic Absorption Spectrophotometer (Shimadzu) was carried out using series of standards of zinc, 0, 0.1, 0.2, 0.3 and 0.4ppm by dilution from stock of 1000ppm AAS zinc standards (Annex 4). Each series of standards were diluted with 5% glycerol to equivalent with viscosity of serum. Zinc concentration was measured using an air-acetylene flame at a wavelength of 213.9 nm and a slit width of 0.7nm. The results were calculated from two runs (Iyengar G.V. 1998 and Peaston R.T. 1973). To minimize the risk of contamination, all glassware and plastic tubes used were immersed in 10% (v/v) solution of nitric acid for 24 h, washed with distilled water and rinsed with deionized water before use. All chemicals used were obtained from Sigma Chemical Company. For serum zinc determination, human serum sample was used as internal control as recommended by IZiNCG (2007) and run in each batch; the result of mean serum zinc was 84.00(±1.8) μg/dl.

The overall design of the steps in measuring serum zinc concentration to assess zinc status for children under five is depicted as follows in Figure 1.

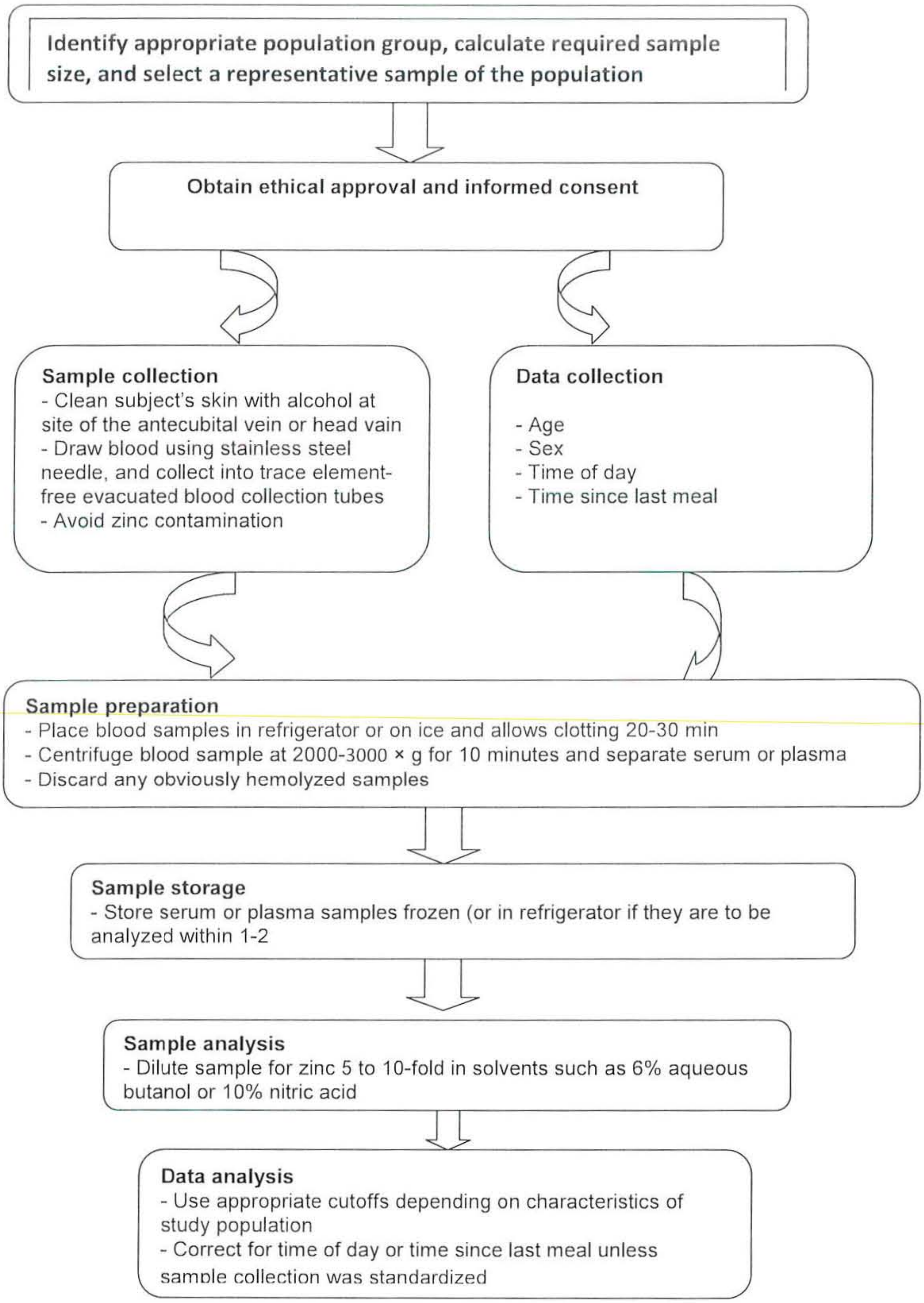


Figure 1. Steps in measuring serum zinc concentration to assess population zinc status (source IZiNCG, Assessing population zinc status with serum zinc concentration, technical brief, No. 2, 2007)

3.9. Statistical analysis

The prevalence of serum zinc deficiency of infants and preschool children was estimated. Zinc deficiency in children was defined as less than 65 μg/dl, as recommended by IZiNCG (IZiNCG, 2007), because blood samples in this survey was obtained not necessarily after fasting. Among 240 samples size, 96% (n=231) of the subjects' blood were collected in the morning between 8-12 am. Blood samples from nine subjects were collected in the afternoon and the cut-off used to define zinc deficiency was adjusted to less than the mean value for blood samples collected in the afternoon (less than 57 μg/dl).

The statistical analysis was carried out using SPSS 16 for Windows. Analysis was carried out at two levels. First, a bivariate analysis was performed to determine the differentials of zinc deficiency under-five by explanatory variables. Pearson's bivariate correlation, independent Student's t-test and ANOVA Post Hoc-Tukey test were performed to test the existence of significant association between zinc concentration and selected risk factors. To control for confounders, linear regression was applied after two models established namely, proximate and distal factors. Anthropometric indices were calculated using ENA SMART 2011 software. The indices are expressed as standard deviation units from the median values of the WHO (2006) standard reference data of US children (WHO, 2006). Infants and preschool children whose height-for-age, weight-for-height and weight-for-age below minus two standard deviations (-< 2Z) from the median of the reference population were considered as stunted, wasted and underweight, respectively.

The family's socioeconomic status (SES) was assessed by interview and observations of housing quality at the time of entry into the selected households. SES data included possessions (e.g., bed, chair, Table, watch, radio, mobile, lamp, cater, horse, donkey, goat and sheep) housing characteristics (e.g., building materials of floor, roof, wall) and agricultural land were included to classify the wealth index of selected households (Schellenberg et al., 2003). The wealth index was calculated based on ownership of selected household assets, size of agricultural land and quantity of livestock. Wealth index quintiles (poorest, poorer, middle, richer, and richest) were computed using Principal Component Analysis (PCA).

The household food insecurity level questionnaires were administered to the mother. A well structured questionnaire was used which was adopted from Food and Nutrition Technical Assistance (FANTA) and evaluated by using Household Food Insecurity Access Scale (HFIAS) on the bases of below and above average of the mean out of nine point scale(Coates et al., 2007).

The categories for dietary diversity were determined by first asking if the mothers had fed their children a particular type of food (30 different types) such as teff, sorghum, millet, maize, barley, rice, cassava, sweet potato, potato, bean, lentil, ground nut, tomato, carrot, leaf vegetable, egg, beef, lamb, goat, chick, fish, milk, lemon, peach, orange, banana, papaya, mango, guava and avocado in the previous 24 hours. These were then categorized into six different food groups namely, grain, root and tuber; legumes and nuts; meat; milk and milk product; egg; vitamin A rich fruits and vegetables.

A score was then calculated to determine how many different types of food groups were consumed by children in the previous 24 hours. This does not measure quantity. Well established food dietary questionnaire were administrated to mothers and evaluated by using Household Dietary Diversity Score (HDDS) (Swindale and Bilinsk, 2006).

3.10. Ethical approval

The study proposal of the main project was presented on the scientific forum of the Ethiopian Health and Nutrition Research Institute and approved at all levels by the Research and Ethical Clearance Committee and McGill University (Canada) Ethical Review Board. The study was also explained to officials of the Zonal Administration, Zonal Health Department of East Gojjam and administrative officials of all Woredas. Data collectors were informed consent or explanatory letter that clarifies the purpose of the study, study confidentiality, and the voluntary right of participation in the study to each child's mother who was eligible to participate and all willing mothers were put their signature on the consent form.

4. Results

4.1. Socio-demographic characteristics

A total of 240 children aged 6-60 months were enrolled from thirteen Woredas of East Gojjam and all infants and preschool children were included in serum analysis. The response rate was 83.3%. In this study all the study areas were rural. The mean child age of the participant was 28.17(±21.57) months.

Of all studied children 105 (43.8%) were female and 135 (56.2%) were male. Infants and preschool children enrolled in the study were categorized into three age groups. Group 1: 6-10 months (37.50% of the total), group 2: 18-22 (29.20% of the total) and group 3: 54-60 (33.30% of the total). Literacy: of the total number of mothers of the selected child 213 (89.10%) were illiterate and 26 (10.10%) were literate and all household heads were farmers. In the study area, the Amharic language, Amhara ethnicity and Orthodox religion followers predominated (Table 1).

Household family members who live on the same land were classified into three groups (small, up to 5 members; medium 6-10 members; and large, 11 or more), and they held 44.20%, 48.70% and 7.10%, respectively. The average number of household members living on the same land was 6.37(±2.99) persons. The wealth index of the participants classified in equal proportion according to the asset they have using PCA. Statistical Figure of socio-demographic and housing characteristics assumed to be associated with zinc status of children is displayed in Table 1.

Table 1. Socio-demographic characteristic of the study subjects East Gojjam, Ethiopia October, 2012

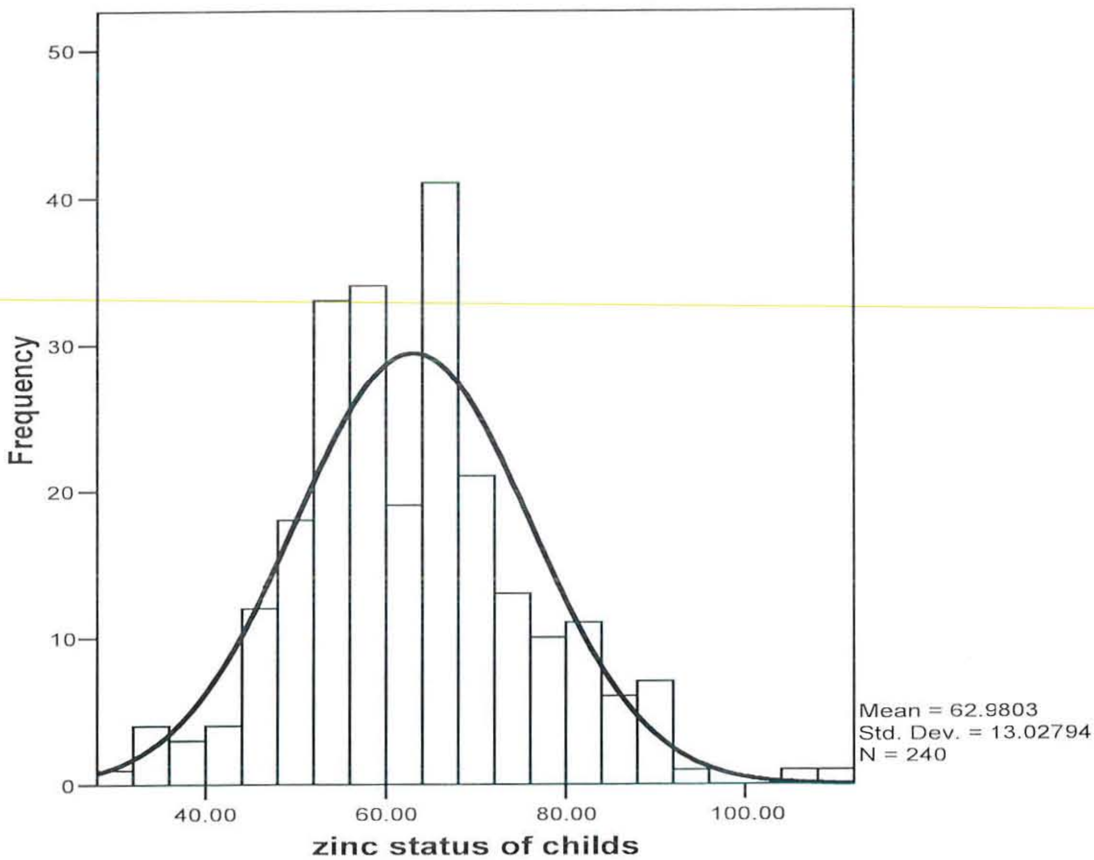
Variables	Frequency	%
Age(Months)		
6-10	90	37.50
18-22	70	29.20
54-60	80	33.30
Sex		
Male	135	56.20
Female	105	43.80
Total no. household members		
1-5	106	44.20
6-10	117	48.70
>11	17	7.10
Literacy of mother*		
Illiterate	213	89.10
literate	26	10.90
Occupation of head of HH**		
Farmer	239	99.60
Other	1	0.40
Literacy of head of HH		
Illiterate	155	64.60
literate	85	35.40
Language		
Amharic	240	100.00
Religion		
Orthodox	234	97.50
Muslim	3	1.30
Others	3	1.30
Wealth index***		
Poorest	47	19.60
Poorer	48	20.00
Middle	49	20.40
Higher	48	20.00
Highest	48	20.00

*Literacy information for one mother was not available, ** HH=household

***Wealth index of the participant household done by using Principal Component Analysis which include possessions (bed, chair, Table, watch, radio, mobile, lamp, cater, horse, donkey, goat and sheep) housing characteristics (building materials of floor, roof, wall) and agricultural land

4.2. Prevalence of zinc deficiency

The mean serum zinc concentration of infants and preschool children was 62.98(±13.01) μg/dl (95% CI: 61.32, 64.63) (Table 2). The value ranged from 28.05 to 108.57μg/dl. Serum zinc level for 6-10, 18-22 and 54-60 months age group were 67.08(±13.21), 61.41(±10.71) and 59.74(±13.62) μg/dl, respectively. The zinc status across age groups were statistically significant (F=7.875, P=0.000). Serum zinc level of the 6-10 months age group was significantly different from 18-22(P=0.015) and 54-60 months age groups (P=0.001). But the 18-22 and 54-60 months age groups mean zinc status were not significantly different (P=0.702) (Table 2).



Zinc status of children expressed in μg/dl.

Figure 2. Distribution curve of zinc status of infant and preschool children in μg/dl aged 6-60 months children in East Gojjam.

Among the study population, 57.10% of infants and preschool children were zinc deficient. The prevalence of zinc deficiency of infants and preschool children for 6-10, 18-22 and 54-60 months age groups were 44.4%, 61.4% and 67.5%, respectively.

4.3. Socio-demographic factors and zinc deficiency

Infants and preschool children's age was negatively associated with zinc status, this means that as the age group increased, the mean serum zinc decreased. The mean serum zinc levels for age 6-10, 18-22 and 54-60 months were 67.08(±13.20), 61.41(±10.71) and 59.74(±13.62) µg/dl respectively. Zinc status among age groups were statistically significant (F=7.875, P=0.000).

The mean serum zinc level for males and female were 62.60(±12.31) and 63.47 (±13.92) respectively. Sex and mean serum zinc status were not significantly associated (t=0.611, P=0.611).

The mean serum zinc level for illiterates and literate mothers' children were 62.81(±13.51) and 64.66(±8.72) µg/dl, respectively, and did not differ significantly (t =0.495, P=0.495).

The total number of family members who lived together on the same land were divided into three groups (small, up to 5 members; medium, 6-10 members; and large, 11 or more) to investigate the effect of family size on the serum zinc levels of children.

There was a significant difference between the total numbers of persons living at the same home and serum zinc level among the study subjects (F=3.501, P=0.032). The mean serum zinc level of the selected child who had 1-5, 6-10 and > 11 household members were 64.44(±11.91), 62.73(±13.72) and 55.59(±13.13) µg/dl, respectively. Mean serum zinc of infants and children who were lived in 1-5 members was different from children who lived with greater than 11 family members (P=0.025), but not differ with children who lived in 6-10 family members.

Data categorizing into five wealth status levels by using principal component analysis, serum zinc concentration of children who belonged to wealth index of poorest, poorer, middle, higher and highest had serum zinc concentration of 61.74(±13.91), 60.64(±12.32), 64.99(±14.40), 64.79(±13.01) and 62.64(±11.32) µg/dl respectively; the groups did not differ significantly (F=0.395, P=0.395).

Table 2. Socio-demographic factors and zinc status of aged 6-60 month's children in East Gojjam Ethiopia, October 2011

Variables	Serum zinc concentration in μg/dl, mean (± SD)	P value
Age(Month)		
6-10	67.08(13.20)[a]	0.000*
18-22	61.41(10.72)[b]	
54-60	59.74(13.63)[b]	
Sex		
Male	62.60(12.31)	0.611
Female	63.46(13.92)	
Total no. household members		
1-5	64.44(11.91)[a]	0.032*
6-10	62.73(13.72)[a, b]	
>11	55.59(13.13)[b]	
Literacy of mother		
Illiterate	62.81(13.51)	0.495
literate	64.66(8.72)	
Food Security		
Above the mean	64.06(13.26)	0.199
Below the mean	61.89(12.79)	
Wealth index**		
Poorest	61.74(13.91)	0.395
Poorer	60.64(12.32)	
Middle	64.99(14.40)	
Higher	64.79(13.01)	
highest	62.64(11.32)	

SD means standard deviation

P value of wealth index total number of household and age were determined by using ANOVA, Post Hoc-Tukey test

P value of Sex, Literacy of mother and food security were determined using Independent-sample T-test

*The mean difference is significant at P= 0.05 level.

** Wealth index of the participant household done by using Principal Component Analysis which include possessions (bed, chair, Table, watch, radio, mobile, lamp, cater, horse, donkey, goat and sheep) housing characteristics (building materials of floor, roof, wall) and agricultural land.
Superscripts of a, b, b indicated that 67 is differ from 61 and 59, but 61 and 59 are not statistically differ.
Superscript a, a, b and b tells us 64 and 62 are not different; but only 64 is different from 55.

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. Each event that took place resulted in a score one but the opposite earned zero. Mean serum zinc status of children who lived in food secure households and children who were food insecure were 64.06(±13.26) and 61.89(±12.79) μg/dl, respectively; the difference was not statistically significant (t=1.412, P=0.199) (Table 2).

4.4. Zinc deficiency and stunting

The analysis of children's nutritional status based on the standard deviation units from the median value for the three anthropometric indices height-for-age, weight-for-age and weight-for-height revealed that 43.30% (95% CI: 37.10, 49.60), 19.70% (95% CI: 15.20, 25.30) and 5.90% (95% CI: 3.60, 9.70) of the total 240 children included in the survey were found to be stunted, underweight and wasted, respectively (Table 3).

Table 3. Anthropometric indices and zinc status of children aged 6-60 months in East Gojjam Ethiopia, October 2012.

Anthropometric indices	Frequency of sex		Total frequency(238)	%	Serum zinc concentration in µg/dl, mean (±SD)	P value
	Male	Female				
Height-for-age						
<-2 Z-score	67	36	103	43.3	63.66(13.21)	0.527
>-2 Z-score	67	68	135	56.7	62.58(12.90)	
Weight-for-age						
<-2 Z-score	32	15	47	19.7	62.94(12.89)	0.869
>-2 Z-score	102	89	191	80.3	63.53(15.60)	
Weight-for-height						
<-2 Z-score	10	4	14	5.9	62.88(13.11)	0.686
>-2 Z-score	124	100	224	94.1	63.74(12.89)	

P value determined by using independent sample Student's t-test

% percent

Males had a higher prevalence of stunting than females (56.3% Vs 43.8%, P= 0.019). Among the three age groups, 6-10 month olds (31.11%) had the lowest prevalence in stunting relative to 18-22 months (61.76%) and 54-60 months (41.25%) age groups (P=0.000). The mean HAZ of infants and preschool children who were aged 6-10, 18-22 and 54-56 months -1.32 (±1.17), -2.19 (±1.06) and -1.84 (±), respectively (F=9.356, P=0.000).

The mean serum zinc concentration of those who were stunted was 63.66(±13.21) μg/dl where as the mean serum zinc concentration of non-stunted subjects was 62.58 (±12.90) μg/dl. The mean concentration of zinc between the two groups were not significant (P=0.527). Also, there were no statistical difference in mean serum zinc status of infants and preschool children who were under weight (P=0.869) and wasting (P=0.686) (Table 3).

4.5. Zinc deficiency and child health

We observed that 14.6% (35/239) of the children presented episodes of fever and 19.3% (46/239) of the children presented episodes of diarrhea during the 14 days preceding enrollment of the day of interview and blood collection in the study. The mean zinc serum level of children with episodes of fever and children with no such episode were 62.62(±14.31) and 63.11(±12.80) μg/dl, respectively); the mean serum zinc level difference between groups was not statistically significant (t=0.200, P = 0.842). The mean serum zinc of children who were presented episodes of diarrhea and children with no such episode were 63.83 (±12.51) and 62.84 (±13.21), respectively. Mean serum zinc of the two groups were not statistically significant (t=-0.478, P=0.644).

Table 4. Child health and zinc status of the study subjects in East Gojjam Ethiopia, October 2012

Variable	Frequency (239)	Serum zinc concentration in μg/dl, mean(±SD)	P value
Fever			
No	204	63.11(12.80)	0.842
Yes	35	62.62(14.31)	
Diarrhea			
No	193	62.84(13.21)	0.644
Yes	46	63.83(12.51)	

P value determined by using Independent sample Student's t-test

SD Standard Deviation

4.6. Zinc deficiency and food diversity

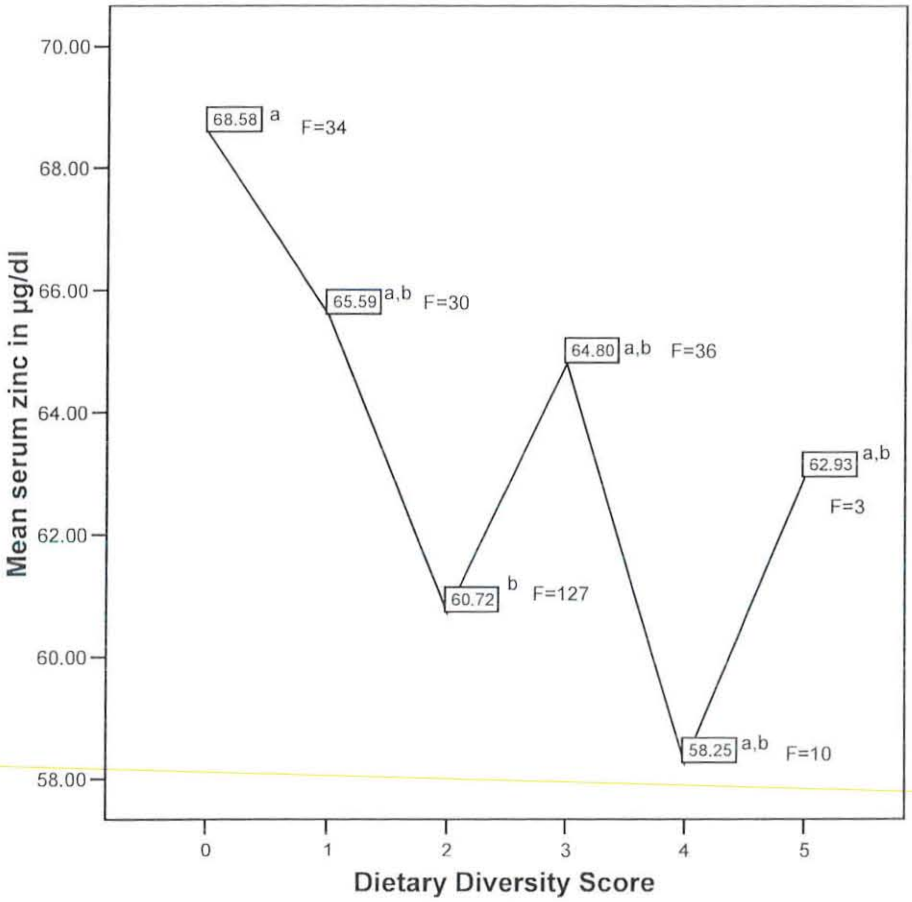
In this study, most of the participants consumed grains (teff, sorghum, millet, maize, barley, and rice) and legume (mostly beans), few participants (n=4) consumed lentil products among six food categories within 24 hours. There was a minimum consumption of vegetables and very few children consumed meat and egg products. Only 17.5% of children had milk. Among the study subjects only 2.5% (6/240) of children consumed meat and egg products.

As shown in Table 5, statistical significances found in mean serum zinc concentration values among infants and preschool children who ate cereal group (t=2.602, P=0.010) and legume products (t=2.230, P=0.027). But, there was no statistical difference in mean serum zinc status of infants and preschool children who ate milk and milk products (t=-0.427, P=0.670), meat (t=0.959, P=0.338), egg (t=0.844, P=0.399) and vitamin A rich foods groups (t=0.687, P=0.493).

Table 5. Dietary diversity and zinc status of children aged 6-60 months in East Gojjam Ethiopia, October 2011.

Variable	Frequency (240)	Serum zinc concentration µg/dl, mean(±SD)	P value
Grains and tubers			
No	36	68.12(11.33)	0.01*
Yes	204	62.07(13.12)	
Legumes and nuts			
No	75	65.74(12.91)	0.027*
Yes	165	61.73(12.92)	
Milk and milk products			
No	198	62.81(13.01)	0.670
Yes	42	63.76(13.31)	
Meat			
No	234	63.11(12.02)	0.338
Yes	6	57.94(14.41)	
Eggs			
No	234	63.01(13.02)	0.399
Yes	6	58.54(7.52)	
Vitamin A rich food			
No	220	63.15(13.01)	0.493
Yes	20	61.06(13.32)	

P value determined by using Independent student's sample t-test
*The mean difference is significant at P=0.05
Grain and tuber: teff, sorghum, wheat, millet, maize, barely, rice and cassava
Legumes and nut: bean, chick pea, lentil and nut
Milk and milk product: milk
Meat: beef, lamb, goat and fish
Vitamin A rich food includes papaya, peaches, tringo, mango, tomato, sweet potatoes and leaf vegetables.



O= exclusive breastfeeding

Dietary diversity score: the categories were determined by first asking if the mothers had fed their children a particular type of food (30 different types) in the previous 24 hours. A score was then calculated to determine how many different type of food groups were consumed by children.

The frequency (F) of infants and preschool children who ate 0, 1, 2, 3, 4 and 5 food groups were 34, 30, 127, 36, 10 and 3, respectively.

Superscript a, b and a, b, tells us only 68 is different than 60, but 68 and 65, 64, 62, 58 are not different.

Figure 3. Dietary diversity score and zinc status of aged 6-60 months children in East Gojjam Ethiopia, October 2011

As shown in Figure 3 above the subjects who consumed exclusive breast feeding had a better zinc status than the children who consumed two food groups (P=0.020). There were no different between zinc status among infants and preschool children who were breastfed and ate one, three, four and five food categories.

Table 6. Linear regression model analysis of distal/ or proximal model of children aged 6-60 months in East Gojjam, October 2011.

Model	Independent Variables	Unadjusted			Adjusted			Adjusted r square
		Unstandardized Coefficient	t	P	Unstandardized Coefficient	t	P	11%
Distal/ or proximal factors	Literacy of mother	1.852	0.683	0.495	0.339	0.362	0.718	
	Number of family	-0.779	-0.672	0.006*	-0.788	-2.803	0.006*	
	Food security	-2.174	-1.289	0.199	1.142	0.526	0.599	
	Wealth index	0.594	0.995	0.321	0.008	0.652	0.515	
	Age categorize	-3.705	-3.807	0.000*	-3.555	-3.435	0.001*	
	Sex	0.864	0.509	0.611	1.071	0.630	0.529	
	Height-for-age-Z score	-0.207	-0.319	0.750	-0.744	-1.149	0.252	
	Diarrhea	2.304	1.096	0.274	3.921	1.566	0.119	
	Fever	-0.478	-0.200	0.842	-3.208	-1.143	0.252	
	Dietary Diversity score	-1.848	-2.328	0.021*	-1.793	-1.702	0.09	

Literacy of mother: education status of mother categorized into two: 1=Illiterate or 2=literate.

Number of family: Total number of household members living on family land.

Food security: The level of household food insecurity was assessed using HFIAS and categorized into two groups: above and below the mean of nine point. 1=above 2=below the mean

Wealth index of the participant household done by using Principal Component Analysis which include possessions (bed, chair, Table, watch, radio, mobile, lamp, cater, horse, donkey, goat and sheep) housing characteristics (building materials of floor, roof, wall) and agricultural land.

Age categorized: infants and preschool children categorize into 3 age groups: 1=6-10, 2=18-22 and 3=54-60 mos.

Sex: categorized into male and female, 1=male, 2=female

Diarrhea: yes or no category, the children presented episodes of diarrhea during the 14 days preceding enrollment of the day of interview. 0=no, 1=yes

Fever: yes or no category, the children presented episodes of fever during the 14 days preceding enrollment of the day of interview. 1=no or 2=yes

Dietary diversity score: the categories were determined by first asking if the mothers had fed their children a particular type of food (30 different types) in the previous 24 hours. A score was then calculated to determine how many different type of food groups were consumed by children.

The linear regression model analysis was used to control the confounder in distal and proximate factors. In the distal factor model the number of family members who live on the same land was the main determinant factor in zinc status of infants and children. This factor was significant in both unadjusted and adjusted linear regression analysis but maternal education, food security and economic status were not significant contributors on zinc status of infant and preschool children in both unadjusted and adjusted linear regression analysis.

In proximate factor model dietary diversity was significant before adjusted; it is association was diminished after adjustment. Age was the main factor which influenced zinc status of infant and preschool children. Age was significant in both unadjusted and adjusted linear regression analysis. But, sex, height-for-age, fever, and diarrhea were not significant on zinc status of children.

Table 7. Linear regression final model analysis children aged 6-60 months in East Gojjam, October 2011

Variables	Unadjusted			Adjusted		
	Unstandardized Coefficient	t	P Value	Unstandardized Coefficient	t	P value
Age categorized	-3.705	-3.807	0.000*	-3.674	-3.801	0.000
Total number of HH living on family land	-0.779	-0.672	0.006*	-0.827	-3.069	0.002
Dietary diversity score	-1.848	-2.328	0.021*	-1.262	-1.632	0.104

Age categorized: infants and preschool children categorize into 3 age groups: 1=6-10, 2=18-22 and 3=54-60 mo.

Total number of household living on family land analyzed per unit, not categorized in the final model.

Dietary diversity score the categories were determined by first asking if the mothers had fed their children a particular type of food (30 different types) in the previous 24 hours. A score was then calculated to determine how many different type of food groups were consumed by children.

As shown in the above Table, age and total number of household living on the family land shows significances in the final model but dietary diversity approaches statistical significance in the absence of strong association with serum zinc status of infants and preschool children.

5. Discussion

Zinc deficiency and socio-demographic factors

In this study, among the participants 57.1% of the children were zinc deficient. Many subjects in all age groups had zinc levels below the cutoff point used to define zinc deficiency. The main reason is in most of the rural areas of Ethiopia consumption of animal sources is mostly limited to occasional public holidays, which indicated minimal intake. Thus one can expect a high prevalence of zinc Deficiency in Ethiopia (Getahun et al., 2001 and Brown et al., 2001). Moreover, many studies conducted in developing countries showed that the prevalence of zinc deficiency was higher in rural area than urban area. In a study conducted in Chinese preschool children on micronutrients deficiency and associated socio-demographic factors, there was a significant difference in the mean serum zinc level by preschool location. Children from rural preschools had the lowest level of serum zinc than children from urban (Liu et al., 2011). Moreover, Villalpando et al. had conducted a micronutrient survey on under 12 years old' children. The prevalence of low serum zinc levels in rural area (40.0%) was twice that of their urban counterparts (18.2%); (Villalpando et al., 2003). In addition, a study conducted within the Mexican national nutrition survey revealed that preschool children who were from rural areas had a higher incidence of zinc deficiency (40%) than did urban areas (18%) (Barquera et al., 2003).

There is a lack of agreement on the normal variation in plasma zinc values according to age. Wouwe et al. (1994) found no age-dependent variation in total serum zinc among healthy Dutch infants and children. Likewise, Karr et al. estimated the plasma zinc values in healthy preschool Australian children and found no significant age-dependent variation (Karr, 1997). Also, no association was found between serum zinc status and age among preschool child in peri-urban population in Delhi (Dhingra et al., 2009) and in Mexican children (Villalpando et al., 2003). However, the results of our study showed a significant difference in mean serum zinc concentration among 6-10 months age-group compared to children in the higher age groups (P=0.000). Our result is consistent with the finding in Nepalese children. A cross-sectional study of 1757 cases of acute diarrhea in 6–35-mo-old Nepalese children was investigated and the result showed that the association between plasma zinc concentration and age was significant (P=0.001); the mean serum zinc concentration was lower in children aged greater than two years compared to infants (Strand et al., 2004). Moreover, the study conducted by Ferraz et al. (2007) showed that when the age ranges were stratified into 12-month intervals, children aged ≥ 48 to < 60 months were found to have lower mean zinc levels than younger children of the remaining age ranges. The mean serum zinc status of infant and children were significant with their age groups. In this finding in the proximal model in linear regression analysis, serum zinc level and age were significant. The analysis showed that children who were 18-22 and 54-60 months of age were likely to have 3.67 μg/dl (95%CI: -5.58, -1.77) lower serum zinc than children who were in the 6-10 months age group. This might be due to appropriate breastfeeding practices among younger age-group which might lead to better zinc status. Moreover, in this survey the children's foods was based on cereals and legumes, so, older children had lower plasma zinc concentrations than did the younger children due to an increased intake of zinc inhibitors as the children's diet became more similar to that of the adults.

The mean serum zinc status among male and female children was not statistically different. This findings is consistent in the previous other study in preschool children living in a peri-urban area of Delhi (Dhingra, 2009). There was no significant group-wise difference at baseline among sex and serum status in the study of zinc supplementation with or without copper in young Ecuadorian (Wuehler et al., 2008). Moreover, the study conducted in preschool children on micronutrients deficiency and associated socio-demographic factors in Chinese children; mean serum zinc level among males and females were not significant (Liu et al., 2011). Also, Bitarakwate et al. (2003) conducted a study on serum zinc status of children with persistent diarrhea admitted to the diarrhea management unit of Mulago Hospital, Uganda. There were no sex differences in the distribution of serum zinc between both the healthy children with no diarrhea and in those with persistent diarrhea. Although, the prevalence of deficiency in healthy populations, in Brazil and around the world serum zinc values were for males and for females, with no statistically significant difference between sexes (Ferraz et al., 2007). But, Thurlow et al., (2006) conducted a study on risk of zinc, iodine and other micronutrient deficiencies among school children in Thailand; the result revealed that male sex represented a risk factor for zinc deficiency. A cross-sectional study of 1757 cases of acute diarrhea in 6–35-mo-old Nepalese children was investigated and the result showed that the association between serum zinc concentrations and sex showed a minimal difference. In contrast; Females had a slightly lower mean plasma zinc concentration than did boys (Strand et al., 2004). Moreover, Chen et al. (2007) result finding on elements in the sera of preschool children living in central Taiwan revealed that the serum zinc levels of males were considerably higher than those of females ($P<0.01$). This result was inconsistent with our result in which female and male serum zinc status was not significantly different.

In this study the family size affected serum status of children. The results showed that, the total number of persons living together in the same home or land is associated with serum zinc status of infant and preschool children. The relationship between the total number of persons living together at same home and the serum status of zinc found was confirmed in both unadjusted and adjusted linear regression model. The result may be attributed to the extended family in the Ethiopian society. In the extended family, the mother may not find enough time to care for the child, or might not find healthy balanced food which indicates the deterioration of the quality of life in extended family. Zere and McIntyre (2003) reported the same results when concluded that family size is one of the factors, which affects child's nutritional status. Present results agree also with Reyes et al., (2004), who reported that the greatest protective effect of stunting was found in Mexican children cared exclusively by their mothers. Our results was inconsistent with the findings of Liu J. et al in children from small families (≤3 persons) which had a higher prevalence of low serum zinc than those from large families (41% vs. 34%, $P<0.001$) (Liu et al., 2011). In this study, when family members increased by one, serum zinc status of infant and children is more likely decrease their serum zinc status by 0.83(95% CI:-1.36, -0.30) μg/dl.

Maternal education is important for child health, nutrition and survival since mother in our culture is responsible for child's food. Low maternal education may lead to the risk of zinc deficiency because illiterate mother can exercise inadequate feeding and unhygienic practice while children grownups (Armar-Klemesu et al., 2000). In this finding mothers education did not affect on their children's serum zinc. The improvement of maternal education about nutrition will help to improve the children's zinc status concurrent with socioeconomic development.

A study of child malnutrition and feeding practice in Malawi revealed that socioeconomic and environmental conditions, in addition to the feeding patterns, are important determinants of malnutrition in developing countries (Nyovani and Mpoma, 1997). Thus, the relationships between socioeconomic status, illness and death were observed to be reverse, with morbidity and mortality concentrated in those at the lowest end of the socio-economic scale (Zere and McIntyre, 2003). Many factors seriously affect child growth and nutritional status, especially in developing countries, such as: family size, educational status, parent's job, parents socio-economic status, knowledge about proper nutrition, prenatal care, mother's age, mother's weight and newborn's sex, (Majlesi et al., 2001). Abdalla et al., (2009) reported that socio-economic aspects influencing food consumption patterns among children under age of five in rural area of Sudan. Cross-sectional study was carried out to investigate some of the social and economic factors, which have a direct and indirect effect on feeding patterns and nutritional status of children under age of five. It was concluded that improvement in societal infrastructure, better maternal education and knowledge about good nutrition were needed to maintain the children's nutritional status in several rural areas of Sudan. Even if, zinc deficiency is a widespread nutritional problem affecting populations of low social economic status in both developed and developing countries (Ruz et al., 1997), some studies show that the socioeconomic variables were only weakly associated with plasma zinc concentration in 6–35-mo-old Nepalese children (Strand et al., 2004). Moreover, the finding that in preschool children on micronutrients deficiency and associated socio-demographic factors, in Chinese children, living in a large house is related to a better zinc level but the lack of significance could be due to the fact that a big house could reflect a better economic status and thus more intakes of animal foods (Liu et al., 2011). It is well suspected that superior household economic standing enhances child zinc status. However, in this study household wealth index was not associated to zinc status. The zinc status by economic standing did not significantly differ from each other which might be because all of the participants were from the rural area and there was not enough variation among groups. The wealth index is relative (not absolute) measure of wealth. So, in relatively homogeneous population in the study area it may lack power.

Economic status of family can limit the child feeding pattern of parents, one research conducted in Canada on preschool children in low income community revealed that median serum zinc levels were significantly lower among children from food insecure households, pointing to poorer quality diets compared to those living in food-secure situations (Broughton et al., 2006). Rose and Oliveira studies also witnessed a decrease in the intake of zinc nutrient in preschooler who lives in food insufficient household in America (Rose and Oliveira, 1997). Moreover, food insecurity in its severe forms (moderate and severe) contributed to the reduction of all food groups, further increasing the dietary insufficiency observed in children. Feinberg et al., assessing families with children 2-13 years of age, found an association between food insecurity and lower intake of nutrients than the food allowances (Feinberg et al., 2008). In this study, the mean serum zinc status and food security were not significantly associated.

Zinc deficiency and stunting

The etiology of growth retardation in zinc deficiency is unknown but it is suggested to be related to nutrition and/or genetics or both; however it is believed that in the pre-school age, nutritional factors predominate over genetic factors (Hambidge et al., 1976). Diminished height (stunting) has been described in infants and children with poor zinc nurture (Umeta et al., 2000). In fact zinc deficiency was described as the cause of pronounced growth retardation, dwarfism and impaired sexual maturation in young people living in Egypt and Iran who consumed diets of low zinc bioavailability (Takyi, 2004). Stunting is a cumulative process that can begin in uterus and continues to about three years after birth. Of a total of 240 infants and preschool children included in the survey, 43.3%, 19.7%, and 5.9% were found to be stunted, underweight and wasted, respectively. Among the three age group 6-10 months (31.11%) was the lowest prevalence in stunting relative to 18-22 months age group (61.76%) and 54-60 months (41.25%) (P=0.000). Stunting highly prevalent in the second age group which include 18-22 months age group, this result is consistent with other studies in Ethiopia (Teshome et al., 2009), a national nutrition survey in Ethiopia which was conducted in 2009 (NNBSR 2009/10) and in other developing countries (Kumar et al., 2006). This is because children younger than 22 months of age responded much more rapidly to the improvement than older children. The result of the study highlights the first two years of life as the most critical period for intervention suggesting an urgent need to institute programs which improve the nutritional status of most vulnerable children in the study area beside nutrition education for mothers. Such programs are probably most effective if they are instituted among children in the first three years of life. After a child reaches 2 years of age, it is very difficult to reverse stunting that has occurred earlier. In some studies, associations of stunting and serum zinc status of preschool children was weak, for instance, anthropometric indices were not associated with serum zinc concentrations in the regression models in Nepal children (Strand et al., 2004). Moreover, one study conducted in Ghana is consistent with the above study which observed the correlation between hair zinc levels and height-for-age Z-scores indicated that there was a non- significant and weak association. This perhaps indicated that any growth faltering, particularly stunting, in these children was not strongly associated with zinc deficiency (Takyi, 2004). In addition, Bitarakwate et al., (2003) found no significant association between the anthropometric indices and the levels of serum zinc in Uganda study. Also, Nhien et al., (2008) finding revealed that there was no significant difference in serum concentration of zinc and stunting in rural preschool children of Vietnam. This study is consistent with the previous studies. In this study the mean serum zinc concentration of those who were stunted was 63.66 (±13.21) μg/dl, where as the mean serum zinc concentration of non-stunted subject was 62.58 (±12.90) μg/dl. The mean concentration of zinc between the two groups were not significant (P=0.527). Stunted children are more likely to have lower zinc intake and plasma zinc concentration than non-stunted children (Brown et al., 2002 and Umeta, 2000); however, stunting is a long-term cumulative effect of malnutrition but the serum zinc status is reflects recent zinc status of children.

Zinc deficiency and child health

The concentration of plasma proteins may be reduced because of increased protein catabolism, reduced synthesis during infection, and intestinal protein loss during diarrhea. The plasma albumin concentration was strongly associated with the zinc concentration (Strand, 2004).

A study by Bitarakwat et al. in Uganda and Strand et al. in Nepal reported a decline in zinc concentrations in serum of children with persistent diarrhea was significantly lower than that of children without diarrhea (Bitarakwate et al., 2003, Strand et al., 2004). Several factors other than the diet and total body zinc affect the plasma zinc concentration (Brown, 1998; Halsted and Smith, 1970). Evidence from human and animal studies shows that plasma zinc decreases during infection (Mwangi, et al., 1995) and that the magnitude of change is related to the severity of infection (Beisel, 1977 and Strand, 2004). According to WHO and UNICEF (2004) report, zinc deficiency plays serious role as the cause of diarrhea and agree with their recommendations to use of zinc supplementation for the treatment of childhood diarrhea. Concerning the relationship between duration of diarrheal attack and serum zinc status, it was found that, there is a significant relationship. As reported in some studies, presence of an inflammatory process may alter zinc homeostasis, usually reducing zinc serum levels (Wieringa et al., 2002). Borges et al., (2007) conducted research on association between serum concentrations of minerals and diarrhea among low-income children in the metropolitan region of Rio de Janeiro. They observed significantly lower zinc serum levels in children that had reported diarrhea episodes 14 days before admission to the study than the children without that report. In our study, however, presence of diarrhea and/or fever (used here as markers of inflammation) during the 14 days preceding the child's admission to the study was not associated with significant changes in zinc serum levels. This result is consistent with Ferraz et al., (2007) in Brazilian children study where there was no strong correlation between serum zinc status of child and occurrence of diarrhea/ or fever at the time of child enrollment. This was probably due to the fact that, at the time of data collection, the survey regarding presence of fever and/or previous episodes of diarrhea was conducted using an open interview depending on more accurate recall by parents, which is not always possible and which may cause some bias in data collection.

Zinc deficiency and food diversity

Millions of people throughout the world may have inadequate levels of zinc in the diet due to limited access to zinc-rich foods (animal products, oysters and shellfish) and the abundance of zinc inhibitors, such as phytates, common in plant based diets (Sandstead, 1991). Individual zinc status is influenced by intake of zinc from the diet, its absorption (or bioavailability) and the loss of it from the body. Kapur et al., (2005) investigated the dietary intakes and growth pattern of children aged 9-to-36 months in an urban slum in Delhi. The results showed that the intake of cereals, roots, green leafy vegetables, other vegetables, fruits, sugar, fats and oils among children was grossly inadequate. The evidence from the study provides a strong basis to suggest low food intake as the main cause of malnutrition and growth retardation in early childhood in poor communities. Moreover, Kapil et al., (2003) assessed the status of serum zinc status among tribal population in India. It was reported that 52.9% of tribal population had deficiency of zinc as revealed by their mean serum level. This was possibly because of low dietary intake of zinc-rich food. The main staple diet was rice and maize. It is known that zinc deficiency is high in populations who consume rice based diets because cereal proteins have low bioavailability of zinc. In East Gojjam community the main staple diet was based on maize, sorghum, wheat, millet, bean, barley and teff. These cereals and legumes have high amount of phytic acid. The bioavailability of these foods is very low. At the time of data collection most children consumed legume and cereal products. Statistical significant relationship was shown among children under five who ate cereal and legume product and those who did not eat them in mean serum status (P=0.01 and P=0.027), respectively. But these significances were diminished in linear regression model in dietary diversity score. Eating legumes and cereals had affect on serum zinc status of children. On the other hand, there was no mean serum zinc difference between children who ate meat; egg and children who did not eat them. This might be the amount and frequency of meat consumption was not enough at the time of data collection. Because, organ and flesh meat and poultry do not contain any known specific anti-nutritional factors that hinder zinc absorption. Eggs and dairy products are also rich in zinc and free of phytates, but they have slightly lower zinc content than can be found in organ and flesh foods (Brown and Wuehler, 2000). Unlikely, root and tuber crops are low in their zinc content, while zinc in cereal staples is most often poorly bioavailable. The bioavailability of zinc in staple cereal and legume is depending on phytate to zinc molar ratio. Phytate: Zinc molar ratios >15 are associated with low bioavailability of zinc. Phytate: Zinc molar ratios of unfermented teff injera, fermented teff injera, unfermented maize injera, unfermented sorghum injera, fermented sorghum injera, fermented wheat injera, maize bread, sorghum bread, wheat bread, maize porridge, sorghum porridge, maize boiled, bean and kale boiled were 28.2, 10.8, 32.6, 38.6, 11.1, 9.4, 37.8, 38.1, 34.4, 32.4, 34.2, 30.3, 33.6 and 17.9, respectively (Umeta et al., 2005). Where diets are plant based and intakes of animal foods are low, the risk of inadequate intake of zinc is very high even when energy and protein intakes meet recommended levels (Urga and Umeta, 2000). Also, infants and preschool children who ate vegetables have lower serum zinc than their counterparts. This is because, among the several factors that interfere with digestion and absorption of dietary zinc, the phytate content of the diet is most important. The higher order phytates, like inositol, hexaphosphate and pentaphosphates found in most cereal, legumes and vegetables, are known to bind to zinc and form poorly soluble complexes that lead to reduced absorption from the intestinal lumen (Krebs, 2000).

Based on the dietary diversity score a child who consumed breast milk had a better zinc status than the children who ate two food groups (P=0.020). In this study the dietary diversity mostly depend on cereal and plant based products with minimum animal products. So, the children's complementary food is prepared predominantly from cereal and legume products, such foods with high phytate may interfere with the absorption of zinc from breast milk (Krebs and Michael, 2007). Moreover, breast milk is the only dietary source of zinc for exclusively breastfed young infants and it remains a potentially important source of zinc for older infants and young children (Brown et al., 2009), that is why in this finding infants had better zinc status than young children who ate at least two food groups. In this study the dietary score was significant when it analyzed with unadjusted linear regression model but it diminished when it analyzed in adjusted linear regression model. This might be due to it running parallel with age and not having a huge sample.

6. Conclusions and Recommendations

6.1. Conclusions

The prevalence of zinc deficiency of infant and preschool children was 57.1%. According to IZiNCG, a zinc deficiency prevalence rate of greater than 20% is considered a public health concern in the area of East Gojjam infants and preschool children. The main determinants of low serum zinc status of infant and preschool children were age and number of family members who living on the same land. Dietary diversity partially influenced on zinc status of infants and preschool children. Child health, food security, economic status, maternal education and anthropometric indices were not found to be associated with zinc status of infants and preschool children. Such potential deficiencies require urgent attention in terms of the endorsement of food fortification program of staple food in long term plan and nutrition education to reduce the phytate from the society staple food like cereals and legumes for short term plan in addition to family planning implementation is recommended.

6.2. Recommendation

Zinc deficiency is prevalent in a community due to low intake of zinc reach food. Direct approaches to increase the zinc status of infant and preschool children should aim at increasing the amount of zinc intake either through dietary diversification, fortification, or reducing the intake of inhibitors of zinc absorption through processing techniques. Dietary diversification to include more micronutrient rich foods in the diet is generally considered to be a sustainable approach in addressing most micronutrient deficiencies. However, in the context of East Gojjam, advocating the increased inclusion of foods rich in bioavailable zinc in the diet will be difficult to achieve since zinc rich foods are usually of animal origin and the relatively high cost of animal source foods make them less accessible to most poor families. In the study area, the consumption patterns are based on cereals and legumes which have high content of phytate, so, processing techniques such as soaking, germination or fermentation at household based phytate reduction technique should be encouraged in these areas since cereal and legume consumption is substantial in these areas. Improving intake of dietary zinc could also be achieved through food fortification, which involves increasing the zinc content of foods through the addition of the mineral to food after processing. Zinc fortification with the use of village based approach has proven to be feasible in certain areas and could also be employed in rural East Gojjam areas to address zinc deficiency at least in young children. In addition to this, nutrition education is very important to mothers or caregivers about preparation of nutrient dense complementary foods for infant. Lastly, family planning implementation also recommended in this area. A limitation of the present study is that no information on acute infection indicator test like C-reactive protein (CRP) and α-1 acid glycoprotein (AGP) which are likely reduced serum zinc concentration due to the redistribution of zinc from serum to the liver.

Reference

Abdalla M, Saad A., Abdullahi H. Tinay E, Khattab A, (2009). Socio-Economic Aspects Influencing Food Consumption Patterns Among Children under Age of Five in Rural Area of Sudan. Pakistan Journal of Nutrition; 8 (5): 653-659.

Alshatwi A.A. (2006). Zinc Status of Preschool Children in Riyadh City. Pakistan Journal of Nutrition; 5: 429-431.

Armar-Klemesu M, Ruel MT, Maxwell DG, Levin CE, Morris SS (2000). Poor maternal schooling is the main constraint to good child care practices in Accra. J Nutr;130:1597–607.

Bahl R, Bhandari N, Hambidge KM and Bahn MK (1998). Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. Am J Clin Nutr; 68:414S–7S.

Barquera S., Rivera J.A, Safdie M., Flores M., Campos-Nonato I.., Campirano F. (2003).Energy and nutrient intake in preschool and school age Mexican children: National Nutrition Survey 1999. Salud Pública Méx; 45(sup 4):540-550.

Beisel W.R. (1977). Zinc metabolism in infection. In: Brewer GJ, Prasad AS, eds. Zinc metabolism: current aspects in health and disease. New York: Alan R Liss: 973–7.

Bilbis L.S, Saidu Y. and Aiyu R.U. (2003). Serum Vitamin A and Zinc levels of Some Preschool Children in Sokoto Metropolis of Nigeria. BIOKEMISTRY; 13: 31-36.

Bitarakwate E., Mworozi E., Kekitiinwa A. (2003). Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda. Afr Health Sci; 3:54 60.

Black R. (2003). Micronutrient deficiency-an underlying cause of morbidity and mortality. Bull World Health Organ; 81:79.

Borges DCV, Veiga Black AP, Santos G B, Oliveira JEF, Barbosa SRF, Moreira S. (2007). Association among serum concentration of minerals, anthropometric indices and diarrhea in low-income children in the metropolitan region of Rio de Janeiro, Brazil. Revista de Nutricao; 20(2):159–69.

Broughton M.A., Janssen P.S., Hertzman C., Innis S. M., Frankish C.J. (2006). Predictors and Outcomes of Household Food Insecurity Among Inner City Families with Preschool Children in Vancouver. CANADIAN JOURNAL OF PUBLIC HEALTH; 97: 3.

Brown K.H. (1998). Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. Am J Clin Nutr;68:425S–9S.

Brown K.H., Peerson J.M., Allen L.H. (1998). Effect of zinc supplementation on children's growth: a meta-analysis of intervention trials. Bibl Nutr Diet ;(54):76–83.

Brown, K.H. and Wuehler S.E. (2000). "Zinc and Human Health: results of recent trials and implications for program interventions and research." Micronutrient Initiative, Canada.

Brown, K.H., Wuehler S.E. and Peerson J.M. (2001). The importance of zinc in human nutrition and
estimation of the global prevalence of zinc deficiency. Food Nutr. Bull., 22: 113-25.

Brown K.H., Peerson J.M., Rivera J., Allen L.H (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr; 75: 1062–71.

Brown K.H., Rivera JA, Bhutta Z, Gibson RS, King JC, Lonnerdal B, Ruel MT, Sandtrom B, Wasantwisut E, Hotz C (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull, 25(1 Suppl 2):S99-203.

Brown K.H., Reina Engle-Stone, Nancy F. Krebs, and Janet M. Peerson (2009). Dietary intervention strategies to enhance zinc nutrition: Promotion and support of breastfeeding for infants and young children. Food and Nutrition Bulletin; 30: S144-S171.

Cameron M.E. and Van Staveren W.A. (1988). Manual on methodology for food consumption studies. Oxford: Oxford University Press.

Castillo C, Vial P, Uauy R. (1988). Trace mineral balance during acute diarrhea in infants. Journal of Pediatric; 113: 452-457.

Chen, Chien-Yi, LIN, Ding-Bang, CHEN, Wen-Kang (2007). Elements in the Sera of Preschool Children Living in Central Taiwan. Chinese Journal of Chemistry; 25, 515—520.

Coates J, Swindale A, Bilinsk P: Household food insecurity access scale (HFIAS) for measurement of household food access: indicator guide version 3. [Accessed on Oct. 2011 http://www.fantaproject.org/downloads/pdfs/HFIAS_v3_Aug07.pdf]

De Onis M., Frongillo E.A., Blossner M., (2000). Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980. Bull World Health Org. 78: 1222-1233.

Dhingra Usha, Girish Hiremath, Venugopal P. Menon, Pratibha Dhingra,Archana Sarkar, and Sunil Sazawal (2009). Zinc Deficiency: Descriptive Epidemiology and Morbidity among Preschool Children in Peri-urban Population in Delhi, India J HEALTH POPUL NUTR; 27(5):632-639.

Donovan U.M. and Gibson R.S. (1995). Iron and zinc status of young women aged 14 to 19 years consuming vegetarian and omnivorous diets. J Am Coll Nutr; 14:463–72.

FAO and WHO, (2002). Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consulation. Rome.

Ferguson EL, Gadowsky SL, Huddle JM, Cullinan TR, Lehrfeld J, Gibson RS (1995). An interactive 24-h recall technique for assessing the adequacy of trace mineral intakes of rural Malawian women: its advantages and limitations. Eur J Clin Nutr; 49:565–78.

Feinberg E, Kavanagh PL, Young RL, Prudent N (2008). Food insecurity and compensatory feeding practices among urban black families. Pediatrics; 122:e854-60.

Ferraz IS, Daneluzzi JC, Vannucchi H, Jordão Jr.AA, Ricco RG, Del Ciampo LA (2007). Zinc serum levels and their association with vitamin A deficiency in preschool children. J Pediatr (Rio J); 83(6):512-517.

Fischer Walker CL, Ezzati M, Black RE (2009). Global and regional child mortality and burden of disease attribuTable to zinc deficiency. Eur J Clin Nutr, 63(5):591-597.

Frankenfield DC, Reynolds HN (1995). Nutritional effects of continuous hemodiafiltration. Nutrition; 11: 388–393.

Getahun Z, Urga K., Ganebo T., Nigatu A. (2001).Review of the status of malnutrition and trends in Ethiopia. *Ethiop. J. Health Dev.*; 15(2):55-74.

Gibson RS (1987). Sources of error and variability in dietary assessment methods: a review. J Can Diet Assoc; 48: 150–5.

Gibson RS (1990). Principles of nutritional assessment. New York: Oxford University Press.

Gibson R.S. (1994). Zinc nurture in developing countries. Nutr Res Rev; 7:151-173.

Gibson RS and Huddle JM (1998). Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. Am J Clin Nutr; 67:702–9.

Gibson RS and Ferguson EL (1999). An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries. Washington, DC: ILSI Press,

Gibson RS, Heath ALM, Limbaga MLS, Prosser N, Skeaff CM (2001). Are changes in food consumption patterns associated with lower biochemical zinc status among women from Dunedin, New Zealand? Br J Nutr; 86: 71–80.

Gibson R.S., Abebe Y., STabler S., Allen R.H., Westcott J.E., Stoecker B.J., Krebs N.F., and Michael K. (2008). Hambidge Zinc,Gravida, Infection,andIron,butNotVitamin B-12 or Folate Status, Predict Hemoglobin during Pregnancy in Southern Ethiopia. J. Nutr. 138: 581–586.

Gibson R.S., Hess S.Y., Hotz C. and Brown K.H. (2008). Indicators of zinc status at the population level: a review of the evidence. British Journal of Nutrition; 99 Suppl. 3: S14–S23.

Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R, Farron M (2000). National diet and nutrition survey: young people aged 4 to 18 years. Volume 1: report of the diet and nutrition survey. London: The Stationery Office,

Halsted JA, Smith JC Jr (1970). Plasma zinc in health and disease. Lancet; 1:322–4.

Hambidge KM., Krebs NF., Walravens PA., (1985). Growth Velocity of young children receiving a dietery zinc supplementation. Nut Res; 1(suppl): 306S-316S.

Haros M., Bielecka M., Honke J., Sanz Y (2008). Phytate-degrading activity in lactic acid bacteria. Sciences Pol. J. Food Nutr. Sci.; 58(1): 33-40.

Hess S.Y , Peerson JM, King J, Brown K.H.(2007). Use of serum zinc concentration as an indicator of population zinc status. Food Nutr Bull,;28:S403-S429.

Hotz C (2007). Dietary indicators for assessing the adequacy of population zinc intakes. Food Nutr Bull 28, S430–S448.

Hunt JR, Matthys LA, Johnson LK (1998). Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk. Am J Clin Nutr; 67:421–30.

International Zinc Nutrition Consultative Group (2004). Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull;25: S91–204.

IZiNCG, (2007). Assessing population zinc status with serum zinc concentration. Technical Brief, No. 2. www.izincg.org. Accessed on Sept. 2011.

Lacey JM, (2007). Zinc-specific food frequency questionnaire. Can J Diet Pract Res; 68: 150-152.

Iyengar GV., (1998). Reevaluation of the trace element content in reference man. Radiat Phys Chem; 51:545–60.

Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS, Bellagio (2003). Child Survival Study Group. How many child deaths can we prevent this year? Lancet; 362: 65–71.

Kapil U, Singh P, Pathak P. (2003). Serum Zinc Levels Amongst Tribal Population in a District of Jharkhand State, India: A Pilot Study. Eastern Journal of Medicine; 8 (2): 33-34.

Kapur D, Sharma S, Agarwal KN, (2005). Dietary intakes and growth pattern of children 9-36 month of age in an urban slum in Delhi. Indian Pediatric Journal; 42: 351-356.

Karr M, Mira M, Causer J, Earl J, Alperstein G, Wood F (1997). Plasma and serum micronutrient concentrations in preschool children. Acta Paediatr; 86:677-82.

Krebs N.F. (2000). Dietary Zinc and Iron Sources, Physical Growth and Cognitive Development of Breastfed Infants. J Nutr; 130: 358S–360S.

Krebs N.F. and Michael K. (2007).Hambidge Complementary feeding: clinically relevant factors affecting timing and composition. Am J Clin Nutr; 85(suppl):639S–45S.

King JC (1990). Assessment of zinc status. J Nutr; 120: 1474–1479

Kumar D, Goel NK, Mittal PC, Misra P (2006). Influence of infant-feeding practices on nutritional status of zinc, and whole body retention of Zn, copper, iron and manganese in rats." British Journal of Nutrition, 34: 243-258 http://www.ocha-eth.org/Maps/downloadables/AMHARA.pdf accessed on Oct, 2011.

Liu J., Yue-Xian Ai, Alexandra Hanlon, Zumin Shi, Barbra Dickerman, Charlene Compher(2011). Micronutrients deficiency and associated sociodemographic factors in Chinese children.World J Pediatr; 7(3):217-223.

Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P (2003). Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics. 112:846-854.

Majlesi, F., Nikpoor B., Golestan B. and Sadre F., (2001). Growth chart study in children under 5 years old in rural area of Khoramabad Province. Iranian Journal of Public Health; 30: 107-110.

Mwangi SM, McOdimba F, Logan-Henfrey L (1995). The effect of Trypanosoma brucei brucei infection on rabbit plasma iron and zinc concentrations. Acta Trop; 59:283–91.

National Nutrition Baseline Survey Report (2010). For national nutrition program for Ethiopia. Ethiopian Health and Nutrition Research Institute.

Nhien N.V., Khan N.C., Ninh N.X, Huan P.V, Hop L. T., Lam N. T., Ota F, Yabutani T., Hoa Vu.Q., Motonaka J., Nishikawa T. and Nakaya Y. (2008). Micronutrient deficiencies and anemia among preschool children in rural Vietnam. Asia Pac J Clin Nutr; 17 (1):48-55.

Nyovani, J. and Mpoma M., (1997). Child malnutrition and feeding practices in Malawi. Food and Nutrition Bulletin; 18: 190-201.

Ohiokpehai Omo, Dorcus Mbithe David and Joyce Kamau (2009). Serum zinc level of school children on a corn-soy blend feeding trial in primary school in suba district. Journal of Applied Bioscience; 17: 904-912.

Olaf Müller, Michael Krawinkel (2005). Malnutrition and health in developing countries. CMAJ;173(3):279-86.

Oyama T, Matsuno K, Kawamoto T (1994). Efficiency of serum cooper/zinc ratio for differential diagnosis of patients with and without lung cancer. Biol Trace Element Res.; 42:115-127.

Pasaoglu H, Muhtaroglu S, Gunes M, Utas C (1996). The role of the oxidative state of glutathione and glutathione-related enzymes in anemia of hemodialysis patients. Clin Biochem.;29:567-572.

Peaston RT., (1973). Determination of copper and zinc in plasma and urine. Med Lab Technol; 30:249.

Prasad AS (2003). Zinc deficiency has been known for 40 years but ignored by global health organizations. BMJ;326:409–10.

Ramakrishnan U. (2002). Prevalence of Micronutrient Malnutrition Worldwide. Nutrition Reviews , Vol. 60, No. 5

Reyes H, Perez R, Sandoval A, (2004). The family as a determinant of stunting in children living in conditions of extreme poverty. BMC Public Health; 10: 4-57.

Rose D. and Oliveira V. (1997). Nutrient Intakes of Individuals from Food-Insufficient Households in the United States. American Journal of Public Health; 87:12.

Rutishauser HE (1973). Food intake studies in pre-school children in developing countries: problems of measurement and evaluation. Hum Nutr;27: 253–61.

Ruz M, Castillo-Duran C, Lara X, Odocro J, Rebolledo A, Atalah E (1997). A 14 Month Zinc Supplementation trial in apparently healthy Chilean preschool children. Am J Clin Nutr.; 66(6):11406-13.

Sandstead H.H., (1991). Zinc Deficiency: a public Health problem? Am J Dis Child; 145: 853-859.

Schellenberg JA, Victora CG, Mushi A, Savigny D, Schellenberg D, Mshinda H, Bryce J(2003). Inequities among the very poor: health care for children in southern Tanzania. The Lancet 361: 561–6.

Semba RD, Bloem MW (2002). The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr.;56:271–281.

Serra-Majem L, Pfrimer K, Doreste-Alonso J Ribas-Barba L, Sa´nchez-Villegas A, Ortiz-Andrellucchi A. and Henrı´quez-Sa´nchez P. (2009). Dietary assessment methods for intakes of iron, calcium, selenium, zinc and iodine. British Journal of Nutrition; 102: S38–S55.

Smolin L.A. and Grosnenor M.B. (2011). Basic nutrition Health eating: A Guide to Nutrition. Chelsea House publishers; 8: 150-159.

Srikumar TS, Johansson GK, Ockerman PA, Gustafsson JA, Akesson B (1992). Trace element status in healthy subjects switching from a mixed to a lactovegetarian diet for 12 mo. Am J Clin Nutr;55:885–90.

Strand TA, Adhikari RK, Chandyo RK, Sharma PR, Sommerfelt H (2004). Predictors of plasma zinc concentrations in children with acute diarrhea. Am J Clin Nutr; 79:451-6.

Swindale A, Bilinsk P (2006): Household dietary diversity score (HDDS) for measurement of household food access: indicator guide version 2. [Accessed on sep. 2011 http://www.fantaproject.org/downloads/pdfs/HDDS_v2_Sep06.pdf]

Takyi E.E.K. (2004). Hair zinc status and its correlation with height indicator in pre-school and school children from a mixed income, low density (mild) community in southern Ghana. East African Medical Journal Vol. 81 No. 1

Teshome B., Kogi-Makau W., Getahun Z. and Taye G. (2009). Magnitude and determinants of stunting in children under five years of age in food surplus region of Ethiopia: The case of West Gojam Zone. Ethiop. J. Health Dev ;23(2):98-106.

Thurlow RA, Winichagoon P, Pongcharoen T, Gowachirapant S, Boonpraderm A, Manger MS, (2006). Risk of zinc, iodine and other micronutrient deficiencies among school children in North East Thailand. Eur J Clin Nutr.;60:623-32.

Umeta M, West CE, Haidar J, Deurenberg P, Hautvast JG (2000). Zinc supplementation and stunted infants in Ethiopia: a randomized controlled trial. Lancet; 355:2021–6.

Umeta M, Clive E, West, Habtamu Fufa (2005). Content of zinc, iron, calcium and their absorption inhibitorsin foods commonly consumed in Ethiopia. Journal of Food Composition and Analysis; 18: 803–817.

UND (2004). Assessment of the risk of zinc deficiency in populations. Food and Science Bull; 25(suppl)2: 130S-161S.

Urga K, Umeta M. (2000). Effects of polyphenols and phytic acid on iron bioavailability from teff as measured by an extrinsic radio iron (59Fe)-tag method. Ethiop J Health Sci; 10:111-12.

Vallee BL, Falchuk KH (1993). The biochemical basis of zinc physiology. Physiol Rev.;73:79-118.

Villalpando S., Garcia-Guerra A., Ramirez-Silva C.I., Shamah-Levy T., Rivera J.A. (2003). Iron, zinc and iodide status in Mexican children under 12 years and women 12-49 years of age. A probabilistic national survey. Salud publisher Mexico; 45: S521-S525

Walravens P.A., Chakar A., Mokni R., Denise J. and Lemonnier D. (1992). Zinc supplementats in breastfed infants. Lannet. 340: 683-685.

Walravens P.A, Krebs NF, Habidge KM (1983). Linear growth of low income preschool children receiving a zinc supplement. Am J Clin Nutr; 38: 195-201.

Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal (2002). Estimation of the effect of the acute phase response of indicators of micronutrient status in Indonesian Infants. J Nutr.;132:3061-6.

WHO (1995). Physical status: the use and interpretation of anthropometry. Technical report series No. 884. World Health Organization, Geneva, Switzerland.

WHO (1997). WHO global database on child growth and malnutrition. Geneva: World Health Organization.

WHO (1998). Complementary feeding of young children in developing countries: a review of current scientific knowledge. Geneva: WHO, (WHO/NUT/98.1).

WHO (2001): Reducing risks, promoting healthy life. Geneva, World Health Organization, 2001 .

WHO (2006). Child growth stander, training course on child growth assessment, version 1. www.izincg.org.

WHO and UNICEF (2004). WHO-UNICEF Joint statement on the clinical management of acute diarrhea. World Health Assembly. Geneva.

WHO / UNICEF / IAEA / IZiNCG (2007). Recommendations for indicators of population zinc status. "Executive summary". Report of Interagency Meeting on Zinc Status Indicators. Food of Nutrition Bulletin; 28: 399-400.

Wouwe V.J.P., Waser I. (1994). Comparison between total and ultrafiltrable serum zinc as test to diagnose zinc deficiency in infants and children. Biol Trace Elem Res; 40:203-11.

Wuehler S.E., Sempértegui F., and Brown K.H. (2008). Dose-response trial of prophylactic zinc supplements, with or without copper, in young Ecuadorian children at risk of zinc deficiency. Am J Clin Nutr;87:723–33.

Yeung L.D. and Laquatra I. (2003). Handbook of nutrition. H.J. Heinz Company; 5:119-159.

Zere, E. and McIntyre D. (2003). Inequities in under 5-child malnutrition in South Africa. International Journal of Equity Health, 2 (7): 75-76.

Annex 1 map of Amhara region

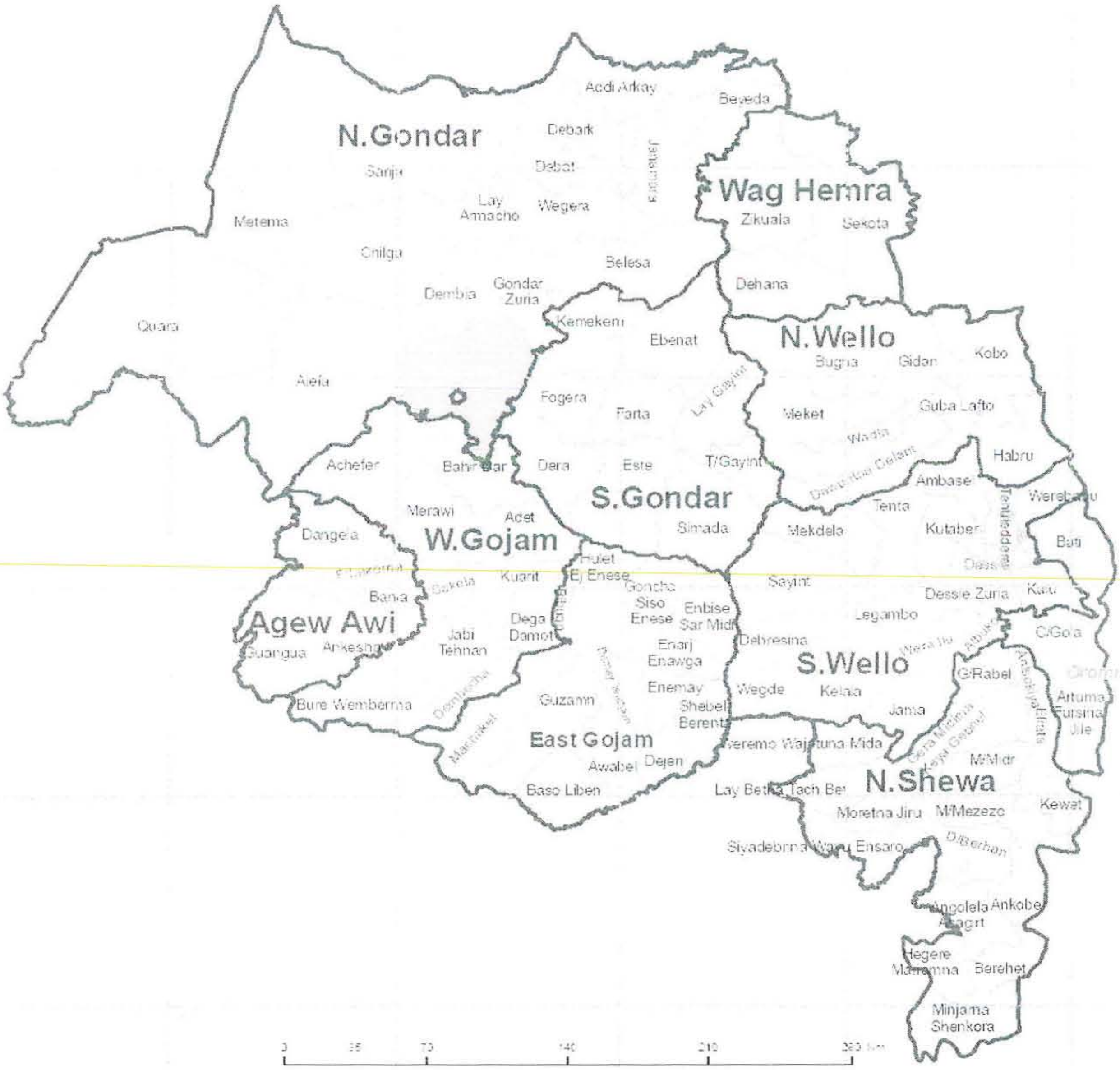


Figure 1 Map of Amhara region. (Source, http://www.ochaeth.org/Maps/downloadable/AMHARA.pdf)

Annex 2 Consent Form for 12-month longitudinal study

Title: The effects of iodized salt on child development in Amhara region in Ethiopia

Principal Investigators: Grace Marquis, Frances Aboud

Institution: McGill University, Montreal, Canada

Collaborating Ethiopian Institution: Dr Cherinet Abuye, Ethiopian Health and Nutrition Research Institute, Faculty of Medicine, Addis Ababa University

Funded by Micronutrient Initiative with head office in Ottawa, Canada and country office in Addis Ababa, Ethiopia

Introduction

Iodine is one of many nutrients that are important for a healthy pregnancy and the normal growth and mental development of infants and children. In many parts of the world, iodine is added to salt to assure that people receive enough in their daily diet. In Ethiopia, people may not get enough iodine from their diet as the food and water are naturally low in iodine. In 2011, the salt producers expect to increase the production of salt with iodine. Market distribution of this salt with iodine will be based on a lottery system in the Amhara region whenever available, until the salt producers can produce enough for everyone. The salt you buy in your marketplace may or may not have iodine in it; the package label will tell you if it has iodine.

If you receive iodized salt in this project, based on average daily salt consumption of Amhara residents, we would expect you to be consuming 59-114 µg of iodine per day from the salt.

The purpose of our study is to test the level of iodine in mothers and their children, to test the level of iodine in the food and water you consume, and examine the physical and mental development of children. Because you have a child of 6, 18 or 60 months of age, we are asking your consent for you and your child to participate in the study.

Procedures

If you agree to participate, we will visit you twice: now and in 12 months. The same measurements will be made each time.

(1) We will ask you questions about your family (including number of members, age, school attendance, housing, water source), that foods that your child eats, your reproductive history (for example, number of pregnancies and children born), your mood and decision-making responsibilities, your child's health, and the child's home environment (for example, play materials). The interview with you will take approximately 30 minutes;

(2) We will take a sample (about a Tablespoon) of the salt that you use daily, because sometimes salt is fortified with iodine.

(3) We will measure the size of your child's thyroid, a part of the neck that processes iodine, as well as take your child's body measurements (for example, weight). In addition, we will also ask your child a series of questions to assess their language, thought processes, and hand and body coordination. This part of the study will take about 45 minutes.

(4) We will collect a small sample of urine (about 1 teaspoon) and take a small sample of blood (10 ml, about 2 teaspoons) to test for iodine, iron, and iodine-related compounds in your child. The urine sample for the 6 mo old will be collected by placing cotton wool in a diaper and waiting for the child to naturally urinate. Finally, if you are breastfeeding your child, we will ask you to provide a small sample (about a Tablespoon) of your breast milk to test for iodine.

Risks

Nothing harmful will come from your participation as all of the tests are routine. The blood sample may cause minor discomfort at the time or a small bruise. The sample is very small and your child's body will quickly replenish it. If your child becomes inattentive, tired or hungry during the tests, we can stop and continue later when it is convenient for you and your child.

Benefits

You will be provided immediately with the results of the hemoglobin test, an indication of the iron status of your child. If the test shows that your child is anemic, you will be referred to the closest health center for treatment. Children who are severely anemic will not be enrolled and appropriate treatment will be assured. There are no other direct benefits to you or your child in participating. However, the findings will possibly help others. If we find that iodine is important for the health and development of your child, then it will support the fortification of salt in Ethiopia.

Cost

There is no cost to you for participating.

Compensation

There will be no compensation for participating.

Participant Rights

If I have said things that are not clear to you, you may ask without hesitation and I will answer. During your interview and that of your child, you may also feel free to ask questions. Your participation is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. Your health centre and extension health worker will continue to provide health services to you as usual. Also you have the right not to answer any questions you do not want to. You may also withdraw from the study at any time.

If in the middle you decide to stop the interview and no longer participate, you can say so without worry.

Confidentiality

Information you provide about yourself, your family, and your child will be kept private. Only the research team will have access to your information. When we write a report, everyone's information will be put together so that information about you or any other individual cannot be seen. Your family will be identified with a number on our forms. A list with the names and numbers will be kept in a private, locked file cabinet.

Persons to contact:

If you have any question, you can ask at any time. If you have additional questions about the study, you may contact: Dr Cherinet Abuye, Ethiopian Health & Nutrition Research Institute, cherinetabuye1@yahoo.com (tel: 251-112-75 63 10) or Dr Grace Marquis, McGill University (Canada) at grace.marquis@mcgill.ca.

If you have questions about your rights as a participant of a research study, you may contact: Chair, Ethiopian Health & Nutrition Research Institute ethics review board (tel: 251-112-75 63 10) or Chair, McGill Research Ethics Board, Faculty of Medicine (Canada) at 1-514-398-3523, or by e-mail researchsec.med@mcgill.ca.

If you agree to participate in the study, please sign or give your left thumb impression at the space indicated below. Thank you for your cooperation.

Signature:

Child's name ______________________________

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

I agree to participate in this study and I agree to have my child participate in this study.

______________________	______________________	______________
Signature or left thumb impression	Printed Name	Date
______________________	______________________	______________
Signature of study representative	Printed Name	Date

Annex 3 Structured Questionnaire to assess the prevalence and risk factors of zinc status among preschool children in East Gojjam, Amhara region, Ethiopia

	Socio-Demographic Information			
No	Questions	Choices		Remark
101	Participants Identification Number (PIN)	W | K | HH | AG | C		
102	Participant address, Woreda, kebele			
103	Religion of the head of household	1. Orthodox 2. Catholic 3. Protestant 4. Muslim 5. If other specify		
104	Main activity of the head of household	1. Farmer 2. Breeder 3. Fisherman 4. Craftman 5. Shopkeeper 6. Peddler 7. If other specify		
105	Literacy of the head of household	1. Illiterate 2. Non formal education		

		3. Formal education		
106	Language of the mother of selected child	1. Amarigna 2. Oromigna 3. Tigrigna 4. If other specify		
107	Literacy of the mother of selected child	1. Illiterate 2. Non formal education 3. Formal education		
		Dwelling		
108	Main material of the roof	1. Natural roofing 2. Rudimentary roofing 3. Finished roofing 4. If other specify		
109	Main material of the floor	1. Natural floor 2. Rudimentary floor 3. Finished floor 4. If other specify		
110	Main material of the walls	1. Natural wall 2. Rudimentary wall 3. Finished wall 4. If other specify		
111	Total number of people from all the household living on the family land	Total in number.............		
		Land Ownership		
112	Does any member of your household own any land that can be used for agriculture?	0. No → 1. Yes		103

113	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		
ASSETS				
No.	question	Coding categories	0-No 1-Yes 2-Yes but not function	Quantity in number
114	Do you have the following assets in your household?	1. Bed 2. Chair 3. Table 4.Watch 5.Mobile phone 6.Nonmobile phone		
		7.Kerosenelamp 8.Electricity 9.Electricmitad 10. Television 11. Refrigerator		
115	Does anyone of this household own the	1. Bicycle 2. Motor cycle 3. Animal drawn cart		

	following?	4. car or truck 5. Boat without motor 6. Boat with motor 7. Irrigation pump		
116	Do you have the following animal in the house hold	1. Cattle 2. Horse/Mule 3. Donkey 4. Camel 5. Goat 6. Sheep 7. chicken		

Information related to Household Food security

No.	Question	0- No		
		1-Yes		
117	In the past four weeks, did you worry that your household would not have enough food?			
118	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?			
119	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?			
120	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?			
121	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?			
122	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?			
123	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?			
124	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?			
125	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?			

Child feeding, Food frequency

No	Food	Did your child ever eat? 0-No 1-Yes	Between yesterday this times to today this time, did	Over the last 30 days, frequency(time your	How many times

			your child eat? 0-No 1-yes	consumption) 1-daily 2- weekly, 3- Monthly 0-not consumed	
126	Teff				
	Sorghum				
	Millet				
	Berbere				
	Maize				
	Wheat				
	Rice				
	Cassava				
	Sweet potato				
	Potato				
	Bean				
	Lentil				
	Groundnut				
	Tomato				
	Carrot				

	Leafy vegeTable				
	Eggs				
	Beef				
	Lamb				
	Goat				
	Chicken				
	Fish				
	Milk/Milk product				
	Guaya				
	Coke (peach)				
	Tringo				
	Lemon				
	Orange				
	Banana				
	papaya				
	Mango				
	Guava				
	Avocado				
Child Morbidity					
127	Did the child suffer the following illness	Diarrhea	0-No 1-Yes		

	in last 14 days?				
		Fever	0-No 1-Yes		
Anthropometric measurement data					
128	Age of subject	___________months			Refer to birth certificate
129	Body Weight of subject	___________kg			
130	Body Height of subject	___________cm			
131	Body mass index of subject	___________kg/m2			
Blood collection form					
132	Sex	1-male 2- female			
133	Time of collection				
134	Time of centrifuge				

Annex 4 Zinc standard calibration curves

Zinc concentration was measured using an air-acetylene flame at a wavelength of 213.9 nm and a slit width of 0.7nm.The results were calculated from two runs (Iyengar GV. 1998 and Peaston RT. 1973). The calibration curve of zinc was depicted as follows.

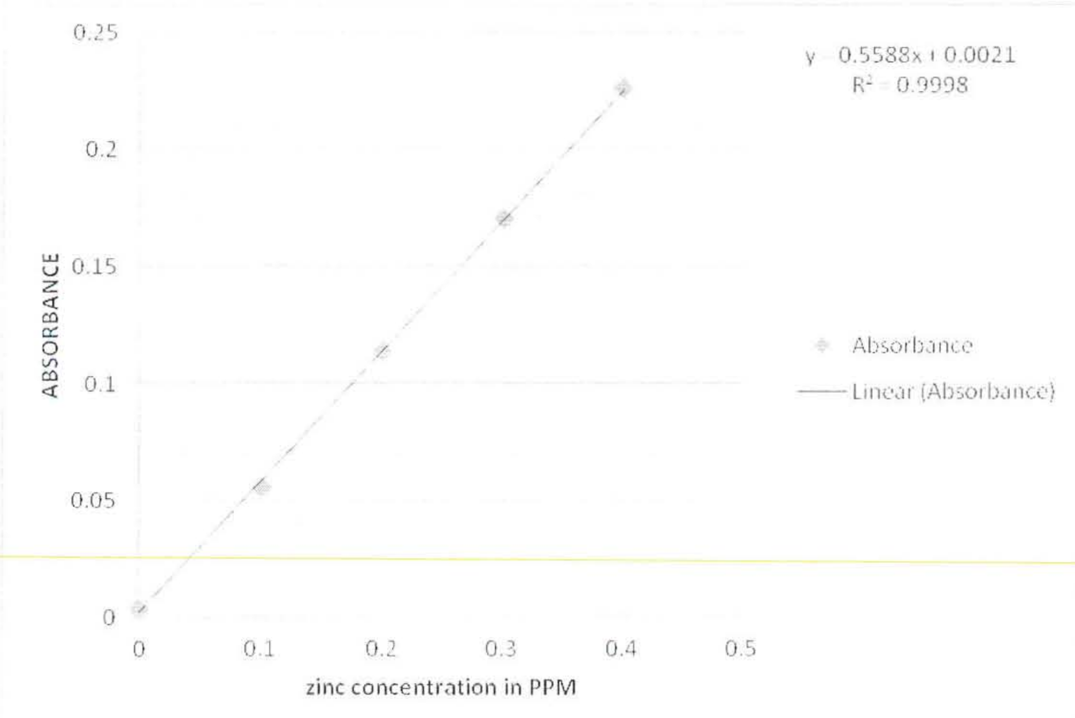


Fig 1 calibration curve of zinc standard using AA6800 Shimadzu AAS

Declaration

I Undersigned, hereby declare that this thesis is my original work, has not been presented for a degree in any other University and all source of materials used for the study have been correctly acknowledge.

Name: Adamu Belay

Signature _______________

Place: Addis Ababa University

Date of Submission_____________

The thesis has been submitted with my approval as supervisors:

1. Name: Grace S. Marquis (PHD)

   Signature________________

   Date ___________________

2. Name: Gulelat Desse (PHD)

   Signature________________

   Date ___________________