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COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Association between dietary diversity score, anemia, iron, zinc and vitamin A biomarkers among women of reproductive age in selected district of west Gojjam and Agew Awi, Amhara Region, Ethiopia

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A thesis submitted to the Center for Food science and Nutrition of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Food Science and Nutrition

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DECLARATION

I, the under signed, declare that this is my original work and that all sources of materials used for the thesis have been dully acknowledged.

Signature ----------------------------- Date ---

The thesis has been approved for submission by:-

Name of supervisor	Signature	Date
Dr.Kaleab Baye (Advisor)	------------------------	----------------------

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This thesis is dedicated to the memory of my beloved brother, Shamble Hassen.

May God Rest your Soul In Peace!

ABSTRACT

Background: Women of reproductive age (WRA) in Sub-Sahara Africa (SSA) are at high risk of inadequate intake of micronutrients due to diet being dominated by starchy staples. Collection of information on dietary diversity to inform food security and nutritional assessments has been promoted, but there is not an agreed upon set of indicators in use. Recently minimum dietary diversity women (MDD-W) endorsed for women, which dictate taking a minimum of five from ten-food groups. However, little is known about MDD-W predictive ability for quality of women's diet in SSA including Ethiopia.

Objective: This study investigated the association between dietary diversity score, anemia, iron, zinc and vitamin A status among women of reproductive age in West Gojjam and Agew Awi zone in Amhara Region, Ethiopia.

Methods: The study conducted in seven kebeles in Bahir Dar Zuria and Dangla District, Amhara Region, Ethiopia. Case control study design employed among anemic and non-anemic women of reproductive age (WRA). About 179 WRA were participated in the study. Data on socio-demographic status and dietary diversity using 24-hr recall, and validated food frequency questionnaires (FFQ) collected.

Results: The prevalence of iron deficiency anemia, zinc deficiency and vitamin A deficiency for both groups in average were18.4 %(serum ferritin < 30μg/L), 65 %(70 μg/L) and was 12.4 %(< 0.81μmol/L), respectively. The overall MDD-W for the WRA was 3.00± 0.757 and the percentage of WRA who achieved minimum dietary diversity (≥ 5 food groups) was only 3%. All biomarkers, except serum transferrin receptor, had positive association with dietary diversity.

Conclusions: The finding of the study indicates high prevalence of iron and zinc deficiencies in both groups of WRA, while from the total, 97 % of WRA failed to fulfill MDD. Low prevalence of vitamin A also observed. There is significant association between DDS and selected biomarkers. Since both groups had low dietary diversity score, MDD-W could not predict the diet quality.

Therefore, programs that focus on improving micronutrient intake through stimulating dietary diversity and fortification of commonly consumed and affordable food products might be needed. In addition to these modern technologies like on-farm diversity, contribute towards improving dietary diversity and quality.

Keywords: Minimum dietary diversity, women of reproductive age, micronutrients

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ACRONYMS

AGA	Alpha- 1-acid glycoprotein
AIDS	Acquired immune deficiency syndrome
CRP	C-reactive protein
CSA	Central statics Agency
DDS	Dietary Diversity score
EDHS	Ethiopian Demographic Health survey
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
EPHI	Ethiopian public Health institute
FANTA	Food and Nutrition Assistance
FAO	Food and agricultural organization
GDP	Gross domestic production
Hb	Hemoglobin
IDA	Iron Deficiency anemia
IQ	Intelligence quotient
MCV	Mean corpuscular volume
MDD–W	Minimum dietary diversity of women
MND	Micronutrient deficiency
SPSS	Statistical package for social science
SSA	Sub-Sahara Africa
STfR	Soluble Transferin Receptor
SZN	Serum zinc
VAD	Vitamin A Deficiency
WDDS	Women Dietary Diversity Score
WHO	World Health organization
WRA	Women of reproductive age

1. INTRODUCTION

1.1. Background

Micronutrient is the umbrella term used to represent essential vitamins and minerals required from the diet in very small amount for normal human cellular and molecular function and development [1, 2]. Though, micronutrients are required in small proportions in a diet, consequences of micronutrient deficiencies (MND) are pervasive, damaging and often irreversible contributing directly or indirectly to morbidity and mortality of billions of people worldwide[1, 3, 4]. Every year hunger and under nutrition claims more than 10 million lives in the world– more than the deaths from AIDS, malaria and tuberculosis combined and many of these people are affected by "hidden hunger," a lack of essential vitamins and minerals [1, 5].

Micronutrient deficiencies are disproportionately affecting Sub-Saharan Africa (SSA) and South East Asian countries due to a diet with limited diversity, poor bioavailability and limited micronutrient content on top of poor hygienic conditions and infections. This place millions of people to remain locked in a vicious cycle of hunger and poverty and the right to food remains the most violated international human right to date despite extensive global economic growth achieved in the past decades [5, 6]. Globally, approximately two billion people, the majority of women and young children, are affected, by micronutrient deficiencies, with even higher rates during pregnancy[7]. Iron, zinc, vitamin A, iodine, folate and other vitamin B deficiencies are among the most widespread global micronutrient deficiencies [8, 9].

Anemia resulting from micronutrient deficiencies such as iron, vitamin A, folate and vitamin B12, infections, and other causes is a major global public health problem[10]. Anemia affects about one fourth of the world's population in both industrialized and developing countries, and its health consequences affect all age groups to varying degrees, with highest prevalence reported in women of reproductive age(WRA), and young children[11]. According to global report of world health organization (WHO) from 1995-2011 prevalence of anemia among women of reproductive age was 29.4%, affecting 528.7 million women [12]. Although it is certainly not the only cause, iron deficiency is the most frequent single cause of anemia and is the most widespread nutrient deficiency in the world [13, 14].

Likewise the potential magnitude of zinc and vitamin A deficiency(VAD) has been recognized as a public-health issue among women of reproductive age (WRA) in SSA and South- East Asia where the diets are predominantly plant-based and animal-source food intakes are low[15]. Economic constraints, socio-cultural limitations, insufficient dietary intake, and poor absorption has been regarded as potential determinants of the prevalence [16, 17]. Precise data on vitamin A and zinc status in non-pregnant and non-lactating is limited in SSA and Ethiopia is no exception despite the prevalence of corresponding micronutrient deficiencies based on inadequate intakes is estimated to be high and serious public health problem [18, 19].

In SSA countries including Ethiopia, many low-income households consume monotonous diets that are of low quality, cereal based, and lacking in vegetables, fruit, and animal-source foods, thereby increasing the risk of micronutrient deficiencies and malnutrition [5, 20]. Consequently, a gap develops between requirement and intake[21]. The groups most at risk to micronutrient deficiencies are WRA, due to their higher nutritional requirements, low dietary intake, improper food storage and preparation ,inequitable distribution of foods with in the household, dietary taboos, infectious diseases, and inadequate care practice , and it is essential to encourage actions to improve their nutrition that may also improve children's health[22-24].

To improve the quality of women's diet, there is a need for high-quality monitoring and evaluation tools in the context of renewed strategies emphasizing dietary diversity to find the best indicators [22]. The vulnerability and gaps in diet quality have been recognized for while though its use is limited due to lack of indicators to allow for assessment, advocacy, and accountability [24]. In meeting held by food and agricultural organization (FAO) and stake holders in 2015, new dichotomous proxy indicator called minimum dietary diversity women (MDD-W) was developed and getting attention to asses micronutrient adequacy of women's diets[25].

It's main use is for assessment of quality of women's diet with specific focus on micronutrient adequacy at national and sub- national levels[26],based on a recall period of a single day and night and reflects consumption of at least five of ten food groups[27]. For improving women's nutrition, progress in designing ,targeting ,and evaluating effective programs there should be a baseline data on women micronutrient intake[24]. Therefore, this study focused on assessing indicators of diet quality of and nutritional status of WRA as well as evaluating the predictive ability of MDD-W in study area [23].

1.2.Statement of the problem

Micronutrient malnutrition remains a substantial cause of morbidity, intergenerational poverty transmission, loss of gross domestic product (GDP), and slow economic progress in many developing countries including Ethiopia [28, 29]. On the average, people suffering from malnutrition could lose about 10% of their possible lifetime earnings[9]. Although a number of countries across the globe have recorded significant progress in reducing the rates of malnutrition over the past two decades, prevalence of anemia, VAD and zinc deficiency in SSA is unacceptably high especially in rural WRA[30]. According to Gebremedhin et al. (2011) more than a quarter of Ethiopian WRA were anemic(27.4%)with reference to the WHO cutoff points [31]. Recent study by Ethiopian Demographic Health Survey (CSA, 2016) covering the whole country reveals an overall 24% prevalence of anemia in WRA. In addition to this women living in rural areas are more likely to be anemic (25%) than those living in urban areas (18%)[32], the magnitude indicates moderate public heath significance of anemia in Ethiopia[33].

Precise data on zinc and vitamin A status in SSA women are lacking; nevertheless, in countries like Ethiopia the prevalence of corresponding micronutrient deficiencies based on inadequate intakes is estimated to be high and low bioavailability of key micronutrients from plant-based diets[34]. Furthermore, studies conducted on dietary diversity of women in Ethiopia, Oromia region[35]and Nigeria, abiate state[36], confirmed that majority of women (85%)consumed either two to three food groups. Staple cereals, pulses and roots/tubers were consumed by 99.6% of the sample [20, 37]. Available compelling scientific evidence demonstrates that dietary diversity is indeed strongly associated with nutrient adequacy and there is no single food, which contains all the required nutrients for optimal health other than breast milk for the first six months of life [20]. Therefore consuming a varied diet is a recommended approach to achieving nutritional requirements and food-based strategies recommended to overcome micronutrient deficiencies.

According to Kennedy,(2009), many organizations promote the collection of information on dietary diversity to inform food security and nutritional assessments, but there is not an agreed upon set of indicators used for this purpose[38]. There is proxy indicator called DDS of infants and young children developed and serving as diet quality indicator of complementary feeding program, it has validated, and a cut-off MDD was set [27]. Recently a similar effort made for women, which dictate taking a minimum of five from ten food groups to be considered adequate. But this was largely from expert opinion and few data gathered from Bangladesh, Philippines and few West African countries[25]. Therefore, the aim of this study was to asses, monitor and evaluate the predictive ability of MDD-W and the extent to which it is associated with anemia, iron, vitamin A, zinc status.

1.3. Significance of the study

Dietary diversification is one of long-term strategies advocated internationally for the improvement of micronutrient intake and status in undernourished individuals [25]. Different studies in several age groups have shown that an increase in individual dietary diversity score related to increased nutrient adequacy of the diet [39]. Though food insecurity is the main problem in developing countries including Ethiopia, further, lack of awareness for benefit of taking more diversified food and lack of knowledge on benefit of nutrients of different foods for them and their children are also cause of micronutrient deficiency especially for WRA[27]. It is important for government and policy makers to know dietary pattern of the study area, prevalence of different micronutrient deficiency before applying strategies for improvement of micronutrient deficiency. So, the information generated from this study used to identify micronutrient status, dietary patterns as well as to evaluate predictive ability of MDD-W in Bahir Dar Zuria and Dangla district, and to draw strategies for improvement of dietary status of rural women in Ethiopia in general and in study area in particular.

1.4. Objective

1.4.1. General objective

- Association between dietary diversity score, anemia, iron, zinc and vitamin A biomarkers among women of reproductive age in selected district of west Gojjam and Agew Awi Amhara Region, Ethiopia

1.4.2. Specific objectives

- To characterize the food consumption pattern of WRA in Bahir Dar Zuria and Dangla district.
- To evaluate the predictive ability of MDD-W
- To assess the prevalence of IDA, zinc and vitamin A deficiency in WRA.
- To determine association between women dietary diversity score and micronutrient status (Iron, zinc, vitamin A).

2. LITERATURE REVIEW

2.1. Background of micro-nutrient Deficiency

Micronutrients is a term often used to include any of more than 20 essential elements required by humans; the elements most commonly studied are calcium (Ca), copper (Cu), iron (Fe),iodine (I),magnesium (Mg), selenium (Se),zinc (Zn),vitamin A, vitamin B12[28]. Micronutrient malnutrition is a global public health problem contributing directly and indirectly to morbidity and mortality of billions of people's worldwide[28]. Globally more than two billion people suffer from a chronic deficiency of micronutrients [40]. The economic consequences of under nutrition in terms of costs to the global economy because of the human capital losses are estimated on USD3.5 trillion per year[41].

Despite the overall progress to reduce global food insecurity and chronic MND in the last few decades, SSA and south East Asia remains the most food-insecure region in the world and close to 800 million people are still classified as chronically hungry[42] and Most of these people are typically deficient in more than one micronutrient[5]. Micronutrient deficiency conditions not only cause specific diseases, but also exacerbate complications of infections, such as measles, tuberculosis and diarrheal diseases, as well as chronic diseases, such as cancers and HIV/AIDS, greatly influencing morbidity, mortality, and quality of life [43].

The adverse health effects of micronutrient deficiencies like impaired physical and cognitive development, decreased immunity, and impaired work capacity and thereby considered hidden hunger, which impact nation economic development are more severe among young children, women of reproductive age, and the developing fetus[43]. Approximately 30% of the world's populations are unable to use their full mental and physical potential as a result of micronutrient deficiencies[44].

In SSA and south East Asia, the majority of the undernourished people live in rural areas and many of them are small-scale subsistence farmers who do not have access to micronutrient rich foods such as fruits, vegetables, animal products and fortified foods, frequently because they are expensive or unavailable[11]. The causes of MND are multiple and interconnected (Figure 2.1). The immediate cause of MNDs is poor nutrient intake through inadequate diets and the other causes may be disease and infestations, in which body's ability to absorb and retain micronutrient decrease with disease. It can even lead to actual losses of them, as in the case of zinc and other minerals loss during diarrhea. Vitamin and mineral nutrition severely compromised by parasitic infestations such as hookworm. The deficiencies caused by diseases leave the individuals more vulnerable to further illness and less able to absorb MNs. The underlying causes of MND are insufficient access to food, inadequate health care, and poor caring practices that inhibit growth and health[45].

Micronutrient deficiency is one of the major public health problems in Ethiopia with women and children most at risk. Dietary inadequacy of consumed nutrients, low bioavailability of key micronutrients from plant based diets and infections are major contributing factors for micronutrient deficiencies in Ethiopia [23]. Malnutrition is a widespread nutrition challenge faced by women living in resource-poor settings, the consequences of which affect not only the health and survival of women but also that of their children, notably through intrauterine growth retardation [26].

Addressing mild to moderate deficiencies of multiple micronutrients may require more comprehensive approach with package of intervention including the right cocktail of micronutrients, whole foods, and entire diets for sustaining the effects [42]. These should be integrated in such a way that there is a smooth transition from the single- or multiple-micronutrient interventions to the most sustainable strategy of dietary diversification [42, 46] and diversified agricultural production to support healthy diets [47].

Given the widespread impacts that MNDs have across the life span, it is not surprising that they cause tremendous financial burdens to societies. Early-life nutrition has long-lasting impacts on the individual and society, including poorer adult health, less educational attainment, diminished work capacity, and lower lifetime earning potential. An estimated 11% of the gross national product in Africa and Asia are lost each year secondary to the high burden of malnutrition [1].

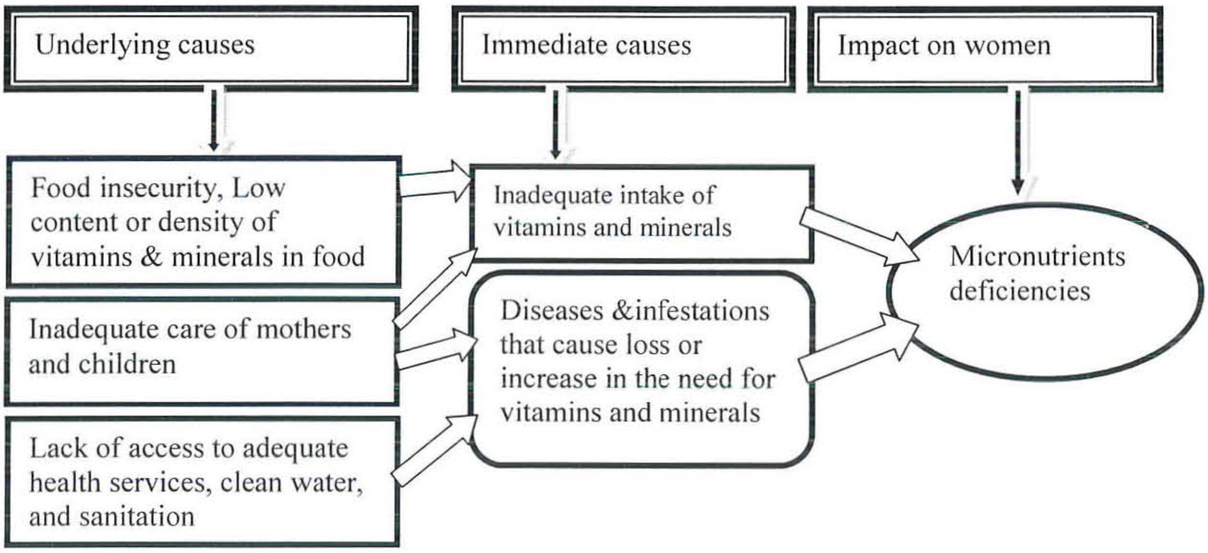

Figure 2.1: Interlinkage among the causes of MNDs

2.2. Prevalence of zinc deficiency

Zinc, a dietary essential trace element, is primarily an intracellular metal involved in numerous metabolic processes, i.e., as a catalyst, structural element, or regulatory ion of gene expression [48]. In the past 40 years, zinc has emerged as a critical nutrient factor for growth, immune function, cognitive development, and normal functioning of the central nervous system[49]. It is required for the activity of more than 100 enzymes involved in most major metabolic pathways and, consequently, is necessary for a wide range of biochemical, immunological, and clinical functions [15].

Because there is no functional reserve or body store of available zinc, except possibly in infants, a regular, adequate dietary supply is required [50]. In developing countries inadequate intake of zinc is by far the most likely cause of ZD, this is because most diets in developing countries have a very low bio-availability of zinc, and at the same time poor sanitation and hygiene generate a high prevalence of recurrent infections such as diarrhea that increase the requirements for zinc[51]. Food items that contain plentiful of zinc include meat (especially liver), seafood and eggs, and thus lacking in many diets. Moreover, although many foods contain zinc at lower levels, the diets in many developing countries also contain many unrefined cereals, resulting in high dietary levels of phytate [15].

Although zinc deficiency is among the most important causes of morbidity in developing-country settings, affects multiple functions in the body [48], Estimates of the prevalence of zinc deficiency and evaluation of its effects on health hampered by the lack of a reliable biomarker of zinc status. Although the WHO, UNICEF, and the International zinc Nutrition Consultative Group jointly recommend the use of serum zinc concentration for assessment of population zinc status, this measure is not reliable at the individual level and few large-scale studies have been conducted in developing countries [48, 52]

Globally, it is estimated that 17.3 -20% (95% CI: 15.9%, 18.8%) of the population could be at risk of zinc deficiency, with the highest estimates in Africa (23.9%) and Asia (19.4%)[48, 53]. In Ethiopia, few studies determined the prevalence of ZD in pregnant women and came up with figures ranging from 53 to 76 %[54]. Worldwide, zinc deficiency is responsible for approximately 16% of lower respiratory tract infections, 18% of malaria, 10% of diarrheal disease, and in total 1.4% (0.8 million) of deaths and the highest risk groups are pregnant women and their young children[53]. Currently, the WHO and UNICEF recommend provision of zinc supplements for 10–14 days along with oral rehydration therapy for acute diarrhea; however, no routine supplementation recommendations currently exist for the prevention of zinc deficiency[1].

An assessment of dietary zinc intakes is the best method for estimating zinc exposure in individuals and populations. Reliable methods developed to evaluate dietary zinc intakes and to assess the risk of inadequacy for individuals and population groups. Although only 1% of the total body zinc is present in circulating blood, several expert committees have endorsed SZCs as a useful biomarker of zinc status, especially for assessing the risk of zinc deficiency in populations[19] .

Moreover, clinical signs of zinc deficiency are clearly associated with a low SZC. Thus, SZC is a biomarker of both zinc exposure and the risk of clinical zinc deficiency. Cutoffs established to identify individuals and populations with an elevated risk of zinc deficiency by using both clinical data and statistical criteria. Current cut-offs for zinc deficiency for non-fasting WRA is <70 μg/dL (10.7μmol/L) (morning) [55].

2.3. Prevalence of vitamin A deficiency

Vitamin A is an essential micronutrient required for the normal functioning of the vision system, immunity, epithelial integrity, cellular differentiation, growth, development and reproduction [56]. World Health Organization (WHO) defines vitamin A deficiency(VAD) as tissue concentrations of vitamin A low enough to have adverse health consequences, even if there is no evidence of clinical deficiency[57]. Globally an estimated 78 to 253 million preschool children are affected by VAD. Pregnant women and WRA also constitute high risk groups for vitamin A deficiency in developing countries[58]. VAD can cause impaired vision and immune function, and may result in preterm birth and infant mortality [59].

The ability of vitamin A to target and affect multiple pathways, as well as its tight homeostatic regulation, makes it a hormone like compound active in a large number of tissues and organs[60]. VAD is a widespread public-health problem with the most vulnerable groups being preschool children[61], WRA, and pregnant women [59].

Serum retinol levels reflect liver vitamin A stores only when they are severely depleted (< 0.7 μmol/g liver) or extremely high (> 1.05 μmol/g liver). The distribution of serum retinol values in a population and the prevalence of individuals with serum retinol values below a given cut-off can provide important information on the vitamin A status of a population and may reflect the severity of VAD as a public health problem[62]. Studies have shown a 1:1 relation between serum retinol and RBP, and therefore, RBP often substituted as an indicator of vitamin A status with the advantage of RBP being more robust for sample collection and handling processes and less expensive to measure [63].

Vitamin A deficiency is of utmost importance as a worldwide nutritional problem, particularly in developing countries[64]. This deficiency is a public health problem that requires intervention when at least one of two specifications is met: (1) the prevalence of low serum retinol is within the range specified by another biological indicator of vitamin A status (including night blindness, breast milk and widespread deficiency is indicated retinol, relative dose–response, modified dose–response or conjunctiva impression cytology); (2) the prevalence of low serum retinol indicates widespread deficiency, and the presence of certain demographic and ecological risk factors[65].

Despite the important consequences of poor vitamin A status, its measurement in human populations is difficult [56, 63] . Although there is gold standard assessment of individual vitamin A status, they are not used for population survey [66]. Therefore, serum retinol and RBP concentrations are surrogate measures of vitamin A status commonly used in nutrition surveys but both biomarkers influenced by the presence of inflammation wherein concentrations transiently decrease during the acute phase response. This results in overestimation of vitamin A deficiency. So, it is recommended to adjust serum retinol and retinol binding protein for inflammation [63] .

2.4. Dietary Diversification

Dietary diversity is well recognized as an important dimension of diet quality and is reflected in all food-based dietary guidelines, usually through daily consumption of recommended food groups. Diverse diets provide micronutrients, phytochemicals and fiber, and satisfy consumer preferences[25]. Available compelling scientific evidence demonstrates that dietary diversity is indeed strongly associated with nutrient adequacy [20, 27]. According to the Food and Agriculture Organization (FAO)[27], dietary diversity is a qualitative measure of food consumption that reflects household access to a variety of foods, and is also a proxy for nutrient adequacy of the diet of individuals[67]. Dietary diversity can be assessed by using tools such as dietary diversity scores and these are good proxies of overall dietary quality and are useful indicators of household food security [68].

Low DDS is more prominent in resource-poor countries like Ethiopia where low-quality monotonous diets are the norm[37]. Women of reproductive age are particularly vulnerable because of their greater micronutrient needs [26, 27, 69, 70]. So dietary diversification is a recommended approach to alleviate nutritional problems resulting from inadequate intake of micronutrient.

Dietary diversification is therefore a key element of diet-based strategies that can be used to increase intake of multiple micronutrients [71].

2.5. Minimum Dietary Diversity-Women (MDD-W)

The 'MDD-W' is a simple proxy indicator recently endorsed to monitor nutrition-sensitive actions and programmes aimed at improving the diet of WRA [26]. According to the MDD-W, women who have consumed at least five of the ten possible food groups over a 24-hour recall period are classified as having minimally adequate diet diversity[72]. The Food and Agriculture Organization (FAO) and the United States Agency of International Development (USAID) both recommend the use of the MDD-W when a categorical indicator of individual dietary diversity for women is needed[73].

MDD-W provides a new tool for assessment, target-setting, and advocacy[69]. The food groups which are included in the MDD-W mostly reflect the diet quality with the probability of minimum micronutrient adequacy of the women's diets summarized across 11 important micronutrients [74]. It is a dichotomous indicator relies on two cutoffs. First, a cutoff chosen for the gold standard indicator of micronutrient adequacy. Second, one needs to select a cutoff for the number of food groups, to examine how well it predicts the status indicated by the gold standard indicator[75]. A woman was classified as having poor DD and food insecure if she had consumed <5 food groups or had achieved MDD-W with good DD and was food secure if she had consumed ≥5 food groups in the previous[25].

The MDD-W is currently one of the key nutrition-sensitive indicators recommended by the FAO [27] and is also proposed by the new initiative of the Gallup World Poll aimed at providing comprehensive data on the quality of people's diets worldwide [69]. The MDD-W responds to several needs including gathering accurate and comparable data on women's diet quality at national or sub national level, making it possible to target at-risk populations, track progress and measure the impact of programs and policies[76].

2.6. Definition of Anemia

Anemia is a disorder which is principally characterized by a decline in the concentration of circulating erythrocytes or hemoglobin in the blood and a concomitant impairment of oxygen transportation [77]. Common etiological classification identifies nutritional, acute and chronic infections, marrow disease and hemolytic anemia types[78]. Nutritional anemia is by far the most common type worldwide and mainly includes iron, folate and vitamin B12 deficiencies and parasitic diseases, such as malaria and hookworm[33]. From all Iron is an essential micronutrient for human health, and is involved in DNA and enzyme synthesis, oxygen transportation, erythropoiesis, metabolism, and immune function. Low iron status may result in iron deficiency anemia(IDA)[79].

Specific physiologic needs vary with a person's age, gender, residential elevation above sea level (altitude), smoking behavior and different stages of pregnancy [80]. It is assumed that IDA considered to be one of the top ten contributors to the global burden of disease [81]. World Health Organization (WHO) classifies Anemia to mild anemia as hemoglobin concentrations of 9.0-10.9 g/dL, moderate anemia as hemoglobin concentrations of 7.0- 8.9 g/dL, and severe anemia as hemoglobin concentrations < 7.0 g/dL[82, 83]. The prevalence of anemia is an important health indicator and when it is used with other measurements of iron status the hemoglobin concentration can provide information about the severity of iron deficiency [12].

2.7. Demography of anemia

The WHO, estimates that anemia affects nearly two billion people all over the world, which is nearly one third of the rapidly growing world population [77]. Despite worldwide economic and scientific development, more than a quarter of the world's population remains anemic and about half of this burden is a result of IDA, which is most prevalent among WRA around 30.2% [84]. On the basis of 2011 global estimates, 43% of preschool children and 33% of nonpregnant women were anemic, with the highest burden in Africa and South Asia[10]. Data collected from 23 countries for pre-school children and non-pregnant women of reproductive age concludes that the proportion of anemia associated with iron deficiency for pre-school children and WRA was 25% and 37%, respectively[85].

World Health Organization (WHO), 2004 estimates, IDA resulted in 273 000 deaths: from this data, 31% are in Africa, all over 97% occurring in low- and middle-income countries. It also caused the loss of 19.7 million disability-adjusted life years, accounting for 1.3% of the global total. Of these lost disability-adjusted life years, 40% were in Southeast Asia, 25% in Africa, and 17% in the Western Pacific. The median annual economic loss because of IDA in ten developing countries including Ethiopia was estimated to be 4% of GDP[84].

2.8. Etiology of Anemia

A systematic analysis of global anemia burden study reported that with a global prevalence of 32.9%, anemia accounted for 9% of the total disability from all conditions in 2010[82]. While the etiology of anemia believed to be commonly iron deficiency, Several other micronutrients were also found to be important factors, yet these associations remain to be confirmed by interventional studies[11]. Even though MND hold the highest share, infections (e.g., intestinal parasites, schistosomiasis, malaria, HIV), and inherited red blood cell disorders (e.g., sickle cell, a-thalassemia) also the cause of anemia [10].

Iron deficiency: The most common nutrition deficiency in both developing as well as in developed countries is iron deficiency, causing approximately 75–80% of the total burden of anemia [82]. Iron deficiency may arise due to an insufficient dietary iron intake. Dietary surveys have shown that, even in developed countries, dietary iron intake is too low in some population groups [83].

Women of reproductive age (WRA) are at particular risk because of menstruation, as pregnancy and childbirth result in additional loss of 580 to 680 mg because of fetal and placental requirements and bleeding during delivery [32]. Ongoing, continuing blood losses predominantly from the gastrointestinal tract due to infections, intestinal parasites, and/or inflammatory bowel disease may contribute to IDA in many parts of the world. Furthermore, women with heavy blood losses at their menstrual periods or by recurrent uterine bleeding due to gynecological disease have a highly increased risk of developing IDA [22].

Vitamin deficiencies: A number of vitamin deficiencies are associated with the development of anemia such as vitamin A and vitamin D deficiency[82]. Although the exact biological mechanisms are yet to be elucidated, vitamin A is known to influence hematopoiesis [56, 58]. The hypothesized mechanisms by which VAD leads to anemia are impaired red blood cell production, increased susceptibility to infections and impaired iron stores mobilization [57, 86]. In humans, cross-sectional studies show positive correlations between serum retinol concentration and Hb that are more apparent with poorer vitamin A status and possibly age[60]. According to different studies VAD may induce anemia by impairing the differentiation and proliferation of pluripotent hematopoietic cells; disturbing renal and hepatic erythropoietin synthesis; reducing mobilization of body iron stores and disturbing iron and haem metabolism[87, 88].

Folate: Folate deficiency causes a specific form of anemia termed megaloblastic anemia[50]. It has been estimated that, in developing countries, folate deficiency occurs in as many as 25% to 72% of WRA [82]. In the fetus and newborn baby, folate deficiency is associated with a high risk of neural tube defects as well as other organ defects. It is present in food of plant origin, e.g., green leafy vegetables, grains, etc. However, the problem is that food preparation procedures (cooking, frying, and milling, baking) actually destroy a high percentage of the folate content in the food [89].

Vitamin B_{12}: Vitamin B_{12} deficiency is probably the second most common vitamin deficiency-causing anemia, which is a megaloblastic anemia with high MCV and typical morphological features such as hyperlobulation of the nuclei of the granulocytes [1]. In developing countries, vitamin B_{12} deficiency constitutes a significant problem, and studies from Lebanon and Turkey have revealed that ~40% of WRA have vitamin B_{12} deficiency which, in addition to insufficient dietary vitamin B_{12} intake [82]. Vitamin B_{12} is present in food of animal origin, which means that subjects consuming food of predominantly plant origin, e.g., vegetarians are not getting enough vitamin B_{12} and therefore are at high risk of deficiency [90].

Infection/infestations: According to different studies, both acute and chronic infections/infestations like intestinal parasites are associated with anemia in many different countries like Ethiopia[82]. Anemia caused by intestinal parasites is mainly due to iron deficiency but is often combined with vitamin B_{12} deficiency as well as folate deficiency [1, 91].

Hemoglobinopathies: In many tropical and subtropical countries, the high prevalence of genetic hemoglobinopathies, thalassemia, sickle cell disorders, etc., plays a significant role in the prevalence of anemia[28]. Geographically, the thalassemia belt covers West and Central Asian countries as well as South-East Asian countries. This is also most common in African, Greek, Italian, Middle Eastern, and Southern Asian populations. In some of the above regions, as many as 30% people may be carriers of the gene defect [92].

2.9. Who are the major risk groups of anemia?

New born and young children: According to reports global anemia prevalence estimates of 47% in children younger than 5 years, in Africa, the prevalence of anemia is estimated to be 65% which unfortunately is on the top of the list [83]. Studies have shown that infants and children born to iron-deficient mothers have a poorer cognitive development of the brain functions and a lower IQ than infants and children born to iron-replete mothers [82, 93]. If iron deficiency is very prevalent in the female population, it may therefore affect the health profile as well as the social structure of the society in a negative direction that is why most countries focused on giving pregnant mothers iron supplementation [91].

Women of reproductive age: On a worldwide scale, more than (30%) 468million WRA suffer from anemia; of these numbers, Africa and Asia account more than 85% of the absolute anemia burden in high-risk groups [59]. Women In developing countries faces a difficult situation concerning their iron balance due to their consumption of a diet containing an insufficient amount of iron to cover their needs, which probably come from non heme iron in part [28, 83].

Pregnant women:. Estimates from the WHO, report that from 35% -75% of pregnant women in developing countries, and 18% of women from industrialized countries are anemic[94]. However, many of these women were already anemic at the time of conception, with an estimated prevalence of anemia of 43% in nonpregnant women in developing countries and of 12% in women in wealthier regions [95]. South- East Asia and SSA present high prevalence of anemia 48% and 57%, respectively. In general, more than 56 million pregnant women all over the world suffer from anemia; of these 87.5% are in more or less developing countries [94].

Postpartum lactating women: If left untreated, anemia in late pregnancy will inevitably continue after delivery into postpartum anemia, which will even be aggravated due to the blood losses at delivery[65]. Lactating mothers from low and middle-income countries are considered as a nutritionally vulnerable group due to frequent pregnancy, caring and nurturing the family as well as workload which intern leads to high maternal mortality [76].

2.10. Health Consequence of anemia

Anemia is an indicator of both poor nutrition and poor health [1]. The most dramatic health effects of anemia, i.e., increased risk of maternal and child mortality due to severe anemia, have been well documented[83, 95]. In addition, the negative consequences of IDA on cognitive and physical development of children, and on physical performance – particularly work productivity in adults are of major concern[2].

I. Reduced Physical and work capacity

Linear relationship has been reported between iron deficiency and work capacity for agricultural workers .Similarly, iron supplementation increased work output among road workers and rubber tappers in Indonesia [82]. Compared with non-anemic women, anemic female workers in China were 15% less efficient in performing their work, which is likely due to reduced oxygen-carrying capacity in an individual's blood[96]. Another study reported that iron supplementation was associated with reduced subjective measures of fatigue and shows improvements in hemoglobin level, that used to increase physical capacity[97].

II. Negative Pregnancy out comes

Anemia has been associated with premature labour and low birth weight and maternal and prenatal mortality[83]. Iron deficiency in WRA increases maternal mortality, prenatal and prenatal infant loss, and prematurity and 40% of all maternal prenatal deaths are linked to anemia[95]. Favorable pregnancy outcomes occur 30-45% less often-in anemic mothers, and their infants have less than one-half of normal iron reserves in developing countries including Ethiopia [98].

III. Morbidity from infection

Due to iron role in several biological mechanisms involved in the immune response to infections Morbidity from infectious disease is increased in iron-deficient populations, because of the adverse effect of iron deficiency on the immune system even though unresolved issues remain to be there [14, 82].

IV. Impaired cognitive development

Iron deficiency can impair cognitive performance at all stages of life, Moreover, the effects of iron deficiency anemia in infancy and early childhood are not likely to be corrected by subsequent iron therapy[99]. An estimated 10-20% of preschool children in developed countries, and an estimated 30-80% in developing countries, are severely anemic at one year of age [78]. These children will have delayed psychomotor development, and when they reach school age they will have impaired performance in tests of language skills, motor skills, and coordination, equivalent to a five to ten point deficit in IQ [94].

2.11. Measurement and assessment of anemia

There are Different diagnosis methods for assessment of anemia but most reliable is Hematologic lab findings[100]. Identification of the cause of anemia is important so that appropriate therapy is used to treat the anemia. According to findings, there are three indicators to measure iron status [82, 101, 102].

1. Hemoglobin measurement

The measurement of Hb is essential for the diagnosis of nutritional anemia and is one of the most common, easiest and least expensive methods but Hb measurement is not very sensitive and specific for iron deficiency (only the third stage affects Hb synthesis). Thus, to determine if iron deficiency is responsible for anemia, it is usually necessary to include other indicators [82].

2. Ferritin measurement

Ferritin is an iron storage protein, regulated post-transcriptionally by cellular iron status via iron-responsive elements in its messenger RNA and is the most important and sensitive indicator of iron deficiency [103]. World Health Organization(WHO) recommends that a serum ferritin concentration <15 μg/L indicates depleted iron stores in those >5 years of age[104]. Measuring SF is the most sensitive and specific test and widely used to identify iron deficiency, but it is spuriously elevated in malignancy, inflammatory conditions or liver disease[10, 31]. SF<15 μg/L in WRA could be diagnosed as iron deficiency. However, results from previous study indicated that if the cut-off of SF level increase to 30 μg/L, the diagnostic accuracy would be improved[101]. The sensitivity would increase from 25 to 92% according, compared with the 15μg/L (cut-off value), and specificity was unchanged (98%)[105]. It is therefore also valuable to measure parameters for acute (CRP and chronic infection [alpha-1-glycoprotein (AGP)] [106].

3. Soluble Transferrin Receptor (sTfR)

The Serum sTfR concentrations indicate the absolute rate of erythropoietin and the adequacy of marrow proliferative capacity for any level of anemia[107]. As the iron supply to the tissues becomes deficient the concentration of sTfR on cell surfaces increases progressively and independently of the presence of adequate iron stores[108]. This means that an increase in serum TfR concentration is a sensitive and quick response to the development of iron deficiency. Conversely, the serum TfR concentration decreases in response to treatment with iron before a change in hemoglobin occurs, so the response to iron can be monitored by changes in serum

TfR[10]. The measurement of sTfR is increasingly being used to determine iron deficiency in situations where infection and inflammation is a factor, as it is much less influenced by this condition[109]. STfR measurements are still much more expensive than ferritin measurements.

4. Total body iron store (TBI)

The logarithm of the ratio of sTfR to SF concentrations is linearly related to total body iron stores (TBI) expressed as mg/kg body weight[110]. TBI is not a measure of the quantity of iron in the individual's body and merely provides a quantitative estimate of the size of the body iron store when iron is present in the store or the size of the functional deficit that would need to be corrected before iron could again be accumulated in the store in an individual who is iron deficient(≤0 mg/kg)[101]. A ratio of sTfR to log ferritin <1 indicates anemia of chronic disease (ACD), whereas values >2 indicate IDA or a mixture of iron deficiency and chronic diseases [14, 109]. The ratio of sTfR to ferritin is the most sensitive indicator for the iron status, since it allows the calculation of the iron stores in mg/kg body weight[111]. It is therefore similar to the gold standard of bone marrow staining in defining iron deficiency [112].

2.12. Prevention and control of micronutrient deficiency

1. Dietary improvement

Food-based approaches represent the most desirable and sustainable method of preventing micronutrient malnutrition even though poverty is the major challenge, Such approaches are designed to increase micronutrient intake through the diet like focusing upon foods which enhance the absorption or utilization of iron such as fruits, vitamin A rich vegetables, and animal products[113]. Finally, effective nutrition education and information on health and nutrition for both supply and demand aspects of programmes may be needed to increase the demand for and consumption of such foods[40]. But poverty is the major challenge to get such to get diversified foods [44]. One of the greatest strengths of it lies in its potential to result in multiple nutritional benefits. These benefits can, in turn, achieve both short-term impact and long-term sustainability. In practice, it should first address the production, preservation, processing, marketing, and preparation of food. Secondly, they should address feeding practices, such as intra-family food distribution and care for vulnerable groups [28].

2. Food fortification

Fortification (enrichment) of food is an effective long-term approach to improving the iron status of populations[114]. Once a fortification programme is established, it is a cost-effective and sustainable means of achieving this purpose [2, 13]. An effective iron fortification programme requires the cooperative efforts of governments, the food industry and consumers in addition to selecting appropriate food vehicles and fortificants [43].

The dietary habits of the population are an important consideration in selecting a food for fortification. For example, possible appropriate food vehicles range from wheat flour or pasta and condiments like sugar, salt [115]. In subsistence farming areas in most developing countries; a fortified-food approach has limited potential because few households ever consume commercially processed foods [77].

3. Iron supplementation

Supplementation is most often used to treat existing iron deficiency anemia[97]. It should also be considered as a preventive public health measure to control iron deficiency in populations at high risk of iron deficiency and anemia [113]. Supplementation programmes, especially for pregnant women, operate in developed as well as in developing countries [93].

4. **Integration with other micronutrient control programmes**

Preventive supplementation is particularly well suited to strategies that combine multiple micronutrient interventions [93]. Accordingly, efforts should be intensively directed to this area. Programmes that involve preparations containing iron, folic acid, and vitamins A and C, directed to infants, children, and pregnant and lactating women, are highly desirable[40]. Similarly, much more attention should be focused on the use of multiple micronutrient-fortified food preparations in supervised feeding programmes[93].

2.13. Interpretation of iron and vitamin A status during an acute phase response

Iron and vitamin A status influenced by infection or trauma, indices of iron status particularly the concentration of SF and sTfR, are influenced by changes in the concentration of acute phase proteins (APPs) during infection or trauma [66]. Inflammation and nutrition are closely related with the result that many nutritional biomarkers, such as serum retinol and RBP, are altered in the presence of inflammation[86]. Two of the most commonly measured APPs to reflect an individual's inflammatory response in cross-sectional surveys are CRP, which rises rapidly and acutely in response to an inflammatory stimulus, and AGP, which has a slower and longer response [63].

The main purpose of the acute phase response is to prevent damage to tissues, and remove harmful molecules and pathogens[116]. During such a response the concentration of some APPs, called positive APPs, increase in the plasma and others, called negative APPs, decrease. The changes in the concentrations of APPs are due largely to changes in their production by hepatocytes, which in turn are regulated by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), which act in a complex network [104].

The acute phase protein (APP) C-reactive protein (CRP) has been used widely to monitor inflammatory processes [105]. Serum concentrations of CRP and other APPs increase rapidly within the first 6 h of the onset of an infection or trauma and reach maximum concentrations between 24 and 48 hours [66]. The concentrations of many serum nutrients or nutritional biomarkers also change rapidly in the same time interval retinol binding protein (RBP), Serum ferritin (SF) and serum zinc [106].

3. MATERIALS AND METHODS

3.1. Study area

This study was conducted from November to December 2017 in Amhara region. Four kebeles were selected from Bahir Dar Zuria Wereda in West Gojjam and three kebeles in Dangla Wereda in Agew Awi zone, Northern Ethiopia that is located about 560 km from Addis Ababa with altitude about 1800-meter elevation above sea level. Based on the 2007 census conducted by the Central Statistical Agency of Ethiopia (CSA, 2007), West Gojjam and Agew Awi zone have a population of 2,106,596 and 982,942 respectively. About 90% of the population lives in rural area. West Gojjam and Agew Awi zones cover 13,311.94-km2 and 9,148.43km ^{2} areas, respectively. West Gojjam bordered in the south by Abay river, that separate it from Oromia and Benishangul-Gumz Region on north by Lake Tana and Abay river which separate it from South Gondar, and in the east by East Gojjam Zone (Figure 3.1). Agew Awi bordered in the west by Benishangul –Gumuz Region, in the north by Semien Gonder zone and in the east by west Gojjam. These zones are relatively food secure area. Agriculture is the main source of income, and sorghum, maize, millet, teff, peas are the main crops cultivated in the area. Livestock is also a significant source of income of the area.

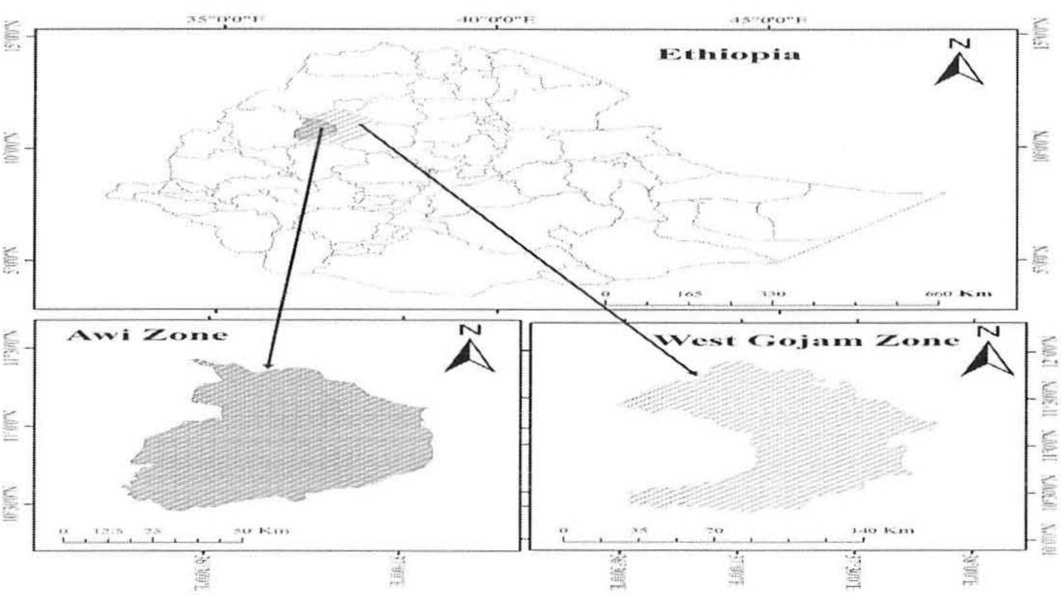

Figure 3. 1Map of the study area

3.2. Study populations

The study population was women of reproductive age (WRA, 15-49 years old) in four Kebeles of Bahir Dar zuria wereda of west Gojjam and three kebeles in Dangla wereda in Agew Awi zone, Amhara Region. The human subjects were selected based on their anemia status. In SSA countries including Ethiopia, 80% of the food supply is provided by smallholder farmers were the majority are, women[117]. Smallholder farmers mostly rely on rain-fed agriculture where fluctuations in rainfall determine the frequency and the amount of harvest. As result, on certain period of the year, food is available in abundance while little is available during the rest[4].Thus, this study was conducted in a season where production was abundance.

3.3. Eligibility criteria

3.3.1. Inclusion criteria

Women of reproductive age (15-49 years old) who, were permanent inhabitants of rural Kebeles of Bahir Dar zuria wereda in West Gojjam and Dangla wereda in Agew Awi zone.

3.3.2. Exclusion criteria

Pregnant and lactating women were excluded from this study, as well as participants who could not come due to illness and other personal reasons during study period.

3.4. Study variables

3.4.1. Dependent variables: hemoglobin, serum ferritin, body iron store (BIS), Serum zinc, serum transferin receptors, retinol-binding protein (RBP).

3.4.2. Independent variables: women's dietary diversity score (WDDS).

3.5. Sample determination

Sample size was calculated using GPower Version 3.1..2 as a tool, this software provides sample size and power analyses for tests that use F, t, chi-square, or z distributions and various distributions for nonparametric applications and to have a sufficient sample size the study was considering medium effect size (0.5); power (1-β) of the study (0.8) and α-error probability (0.05) [118]. By using this software sample of 200 women of child bearing age of which 100 anemic and 100 controls were recruited by taking 10% non- response rates into consideration.

3.6. Sampling techniques

In two wereda of west Gojjam and Agew Awi, zone screening for anemia done using hemocue and randomly 100 anemic and 100 non-anemic WRA selected for further study. Balloting used to select women of reproductive age (15-49years) from different households to obtain a sample size of 200.

3.7. Study Design and sampling approach

Case control study design employed among anemic and non-anemic women of childbearing age. The measuring instruments included a pre-tested socio-demographic and health questionnaire, a validated food frequency questionnaire (FFQ)and 24 hour dietary recall ,were carried out to get purposive and full information [25, 119].

3.8. Ethical consideration

The institutional review boards of the College of Natural and computational Sciences of Addis Ababa University and the Amhara Regional Health Bureau approved the study protocol before embarking on the study. Informed consent sent to the community, the area visited, and participants were enlightened about the reason for conducting the study. Participation in the study was entirely on voluntary basis. Written informed consent obtained from the women who selected for the study in the presence of local kebeles (smallest administrative unit) administrators after a detailed explanation of the purpose and methods of the study.

3.9. Training of research assistants

3.9.1. Food consumption pattern

Research assistants trained on questionnaire administration and on how to conduct interviews, identify the food groups and the individual foods in each food group in order to place recalled foods appropriately into the ten food groups based on 24-hour recall and food frequency questionnaires (FFQ)over period of a week recall was collected.

3.9.2. Anemia measurement

Phlebotomist was knowledgeable of how to draw venous blood as well as conducting laboratory activities to determine malaria and anemic status as well as how to handle serum sample from sample collection area to temporary storage area.

3.10. Data collection

Data collection done by the use of a questionnaire to obtain enough information on socio-demographic and socio-economic characteristics of the rural women and their dietary habits.

3.11. Hemoglobin measurement

A drop of blood from the fingertip was taken using an automated lancet and the first drop of blood removed with sterile cotton swap. Then it was directly collected into the microcuvette.

3.11.1. Calibration of hemocue equipment

Blood sample was collected in tube containing Ethylenediaminetetraaceticacid (EDTA), centrifuged and separated plasma and cells. Mixture of plasma and cells will be prepared to give mass concentration values for hemoglobin of about 50,100,150,200g/L using bland and Altman method[120]. These mixtures were divided into portions and analyzed each portion. The absorbance for EDTA blood samples were analyzed [91].

3.11.2. Blood collection and biochemical analysis

Fasting venous blood (6mL) was collected, and, sample collection sites was previously cleaned with isopropyl alcohol [28]. Blood samples were collected in vacutainer tubes: EDTA-coated tubes for the subsequent analysis of hemoglobin (Hb), untreated tubes for the analysis of zinc, iron biomarkers (serum ferritin, body iron store, soluble serum transferrin receptor)[38]. After 30 minutes, collected blood samples were centrifuged at 3000 rpm for 10 minutes. Then the separated serum was placed in cold box carrier containing ice packs and placed in refrigerator to maintain the temperature around -8^{o}c until transported to Ethiopian Public health institute (EPHI) to be kept in deep freezer at a temperature of −80 °C until subsequent analysis[30, 68].

3.11.3. Analysis of iron biomarkers and retinol binding protein (RBP)

Determination of serum ferritin, sTfR, body iron store (BIS),CRP, AGP, and RBP concentration was conducted using a combined sandwich enzyme-linked immunosorbent assay in German laboratory [8, 50].

3.11.4. Zinc measurement

Micro plasma atomic emission spectrophotometer 4200 was used to measure zinc levels at a wavelength of 213.857 nm by utilizing an air acetylene flame and hollow cathode lamp as a light source. Zinc standard was prepared using 0, 5, 10, 15,20ppm. Four (4) ml Butanol was used to dilute 200μL serum zinc. Zinc deficiency defined as mild to moderate for serum zinc levels 59-70 μg/dL and severe zinc deficiency for levels below 50μg/dL[121].

3.12. Assessment of dietary diversity

Dietary assessment was investigated using the 24-hour recall dietary intake interview and FFQ. The women were asked to recall the foods consumed both within and outside the home in the last 24hours[25]. Dietary diversity was assessed using a new standardized Food and Nutrition Technical Assistance Project (FANTAIII) questionnaire for calculating women dietary diversity score based on ten food groups. The WDD questionnaire ten food groups are: (I) starchy staples (II) beans and peas (III) nuts and seeds (IV) dairy products (V) flesh foods (VI)eggs (VII)Vitamin A-rich dark green leafy vegetables (VIII) other vitamin A rich fruits and vegetables (IX) other vegetables (X)other fruits[122]. The sum of the different food groups summed up to ten points and this indicated the dietary diversity score for an individual women. Women with a dietary diversity score of five and above was classified as having high dietary diversity and those below five has been classified as low dietary diversity[27].

3.13. Statistical analysis and deficiency cut-offs

Anemia was defined according to WHO standards as Hb below 12 mg/dL for non-pregnant women[78].Hb concentrations were adjusted downwards for altitude for all women and children living in villages with an altitude above 1000 m. SF and RBP concentrations were adjusted for inflammation using biomarkers (CRP and AGP) according to methods proposed by Turnham et al. [66, 106]. Corrected values of SF and retinol within inflammation groups were obtained by multiplying values by their respective group corrector factors: SF by 0·77 (incubation), 0·53 (early convalescence) and 0·75 (late convalescence); retinol by 1·13 (incubation), 1·24 (early convalescence) and 1·11 (late convalescence)[65]. A statistical analysis program SPSS version 20 was used to analyze the data. Frequency distribution of socio-demographic characteristics of the study population was determined. Data has been coded and the means and standard deviations (means) was calculated for descriptive data. Bivariate correlation was used to analyze the association between dietary diversity score and micronutrient status[123].The scores obtained from the dietary diversity questionnaire have been compared with standard from the Food and Agricultural Organization. A p-value of less than 0.05 was accepted as statistically significant[124].

4. RESULTS

4.1. Socio-demographic information

Among the 200-subjects in the study, only 179 WRA were completed the study giving response rate of 89.5%, therefore the analysis was based on these figures. The mean age of the participant was 35.5 years (standard deviation=8.29), and it ranges from 15 to 49 years (Table 4.1). About 69% of participants were illiterate while 31% were able to read and write. About 98% participants were also small-scale farmers. Nearly half (49.7 %) of women had monthly income of between 1,000 and 2,000 birr while about 33% of women had monthly income more than 2,000 birr and the other 17% of women had monthly income below 1,000 birr or no formal income .Most (60%) of women have an average of four children. Nearly, 94% of participants had no children below the age of three on the other hand only 30% of participants had no children with age of three to five years. About 85% of participants had an average household size of six. Nine out of ten (92 %) of women were married; other 8% were either widow or divorced.

4.1.1. Types of medication taken and source of water

Among 179 women involved in the study, nearly 45 % of them were taking different medication and out of which 75% of them, were taking oral contraceptives. Nearly half of (56%) participants were getting their source of drinking water from communal pipe water, 26% from surface water, and 18% from spring water (Table 4.2). Furthermore, about 34% use different methods to treat the drinking water, of which 65% use chemical treatment, 15% boiling, 12% straining (cloth), and 8% use other methods. More than two third (69%)of women were getting their drinking water walking less than 10 minutes only; over all it takes less than 30 minute to get drinking water for all households in round trip.

Table 4. 1Socio-demographic characteristics

Variables	**Proportion (%)**
Age	
18-25	14.0
26-33	40.2
35-49	45.8
Educational status	
Illiterate	68.7
Literate	31.3
Marital status	
Married	91.6
Divorced	5.0
Other	3.4
Occupation	
Farmer	97.8
Other	2.2
Monthly income	
≤ 1000 birr	17.3
1000-2000	49.7
≥ 2000	33
Number of children	
1-3	40.3
>4	59.8
Children≤3 years old?	5.6
Children 3-5 years?	70
Children ≥6 years old?	82
Total number of household	
1-3	15
>4	85

Table 4. 2Medication history and source of drinking water

	Proportion (%)
Medication use	45.3
Type of medication?	
Oral contraceptives	75.3
Other medication	24.7
Abortion?	4.5
Source of drinking water	
Pipe water	55.9
Spring water	17.9
Surface water	25.1
Other	1.1
Minute of walking to get drinking water?	
≤20	81.6
20-30	16.2
>30 minutes	2.2
Treat water to make it safer for drink?	33.5
Water treatment techniques	
Boiling	15
Water filters chemicals	65
Strain through the cloth	11.7
Other methods	8.3

4.2. Food consumption based on 24 hours recall results

The diet of study population contained mostly cereals, legume and pulses, dark green vegetables and other vegetables. Out of 84 anemic women, nearly all consumed cereals (99%) and pulses (100%). on the contrary, the consumption of dark green leafy vegetables (34 %), other vegetables (23 %), vitamin A rich vegetables (9.5 %) and nuts (7.9 %) was low. Furthermore, hardly any or no WRA consumed animal and dairy products and other fruits (Figure 4.1). Similarly, dietary pattern was also observed in non- anemic groups, all participants consumed cereals (100 %) and pulses (98%). About half of non- anemic women of them were consumed dark leafy vegetables. The consumption of other vegetables (25 %), vitamin A rich vegetables (19 %) and nuts (7 %) were low. Furthermore, consumption of animal and dairy products, and fruits were negligible (Figure 4.2). Overall, teff, finger millet, oats, maize, sorghum, chickpeas and lentil were the most common consumed cereals and legumes.

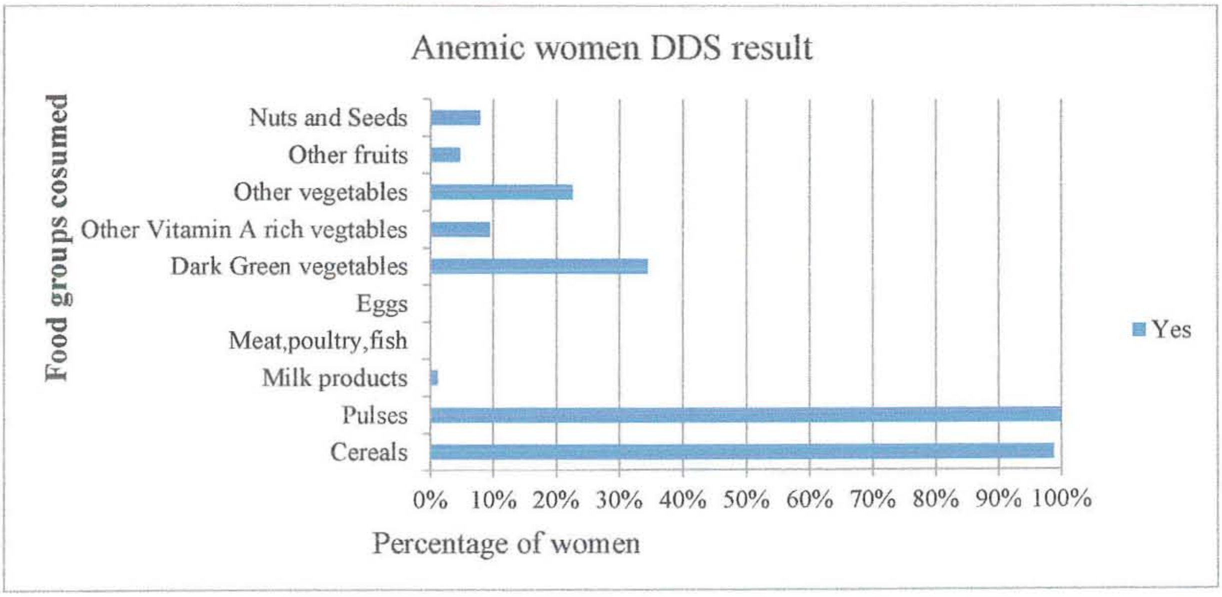

Figure 4.1Food groups consumed according to women's dietary diversity score (WDDS) classification of anemic women.

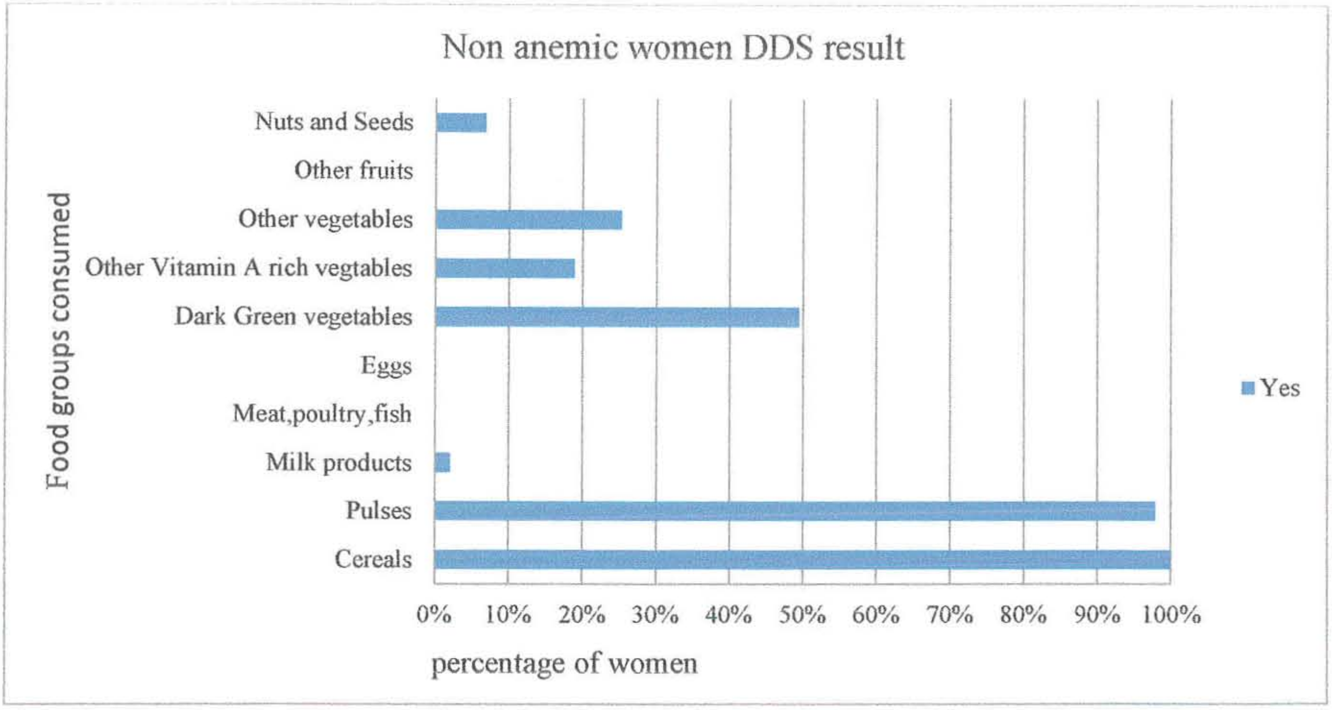

Figure 4. 2.Food groups consumed according to the women's dietary diversity score (WDDS) classification of non-anemic women.

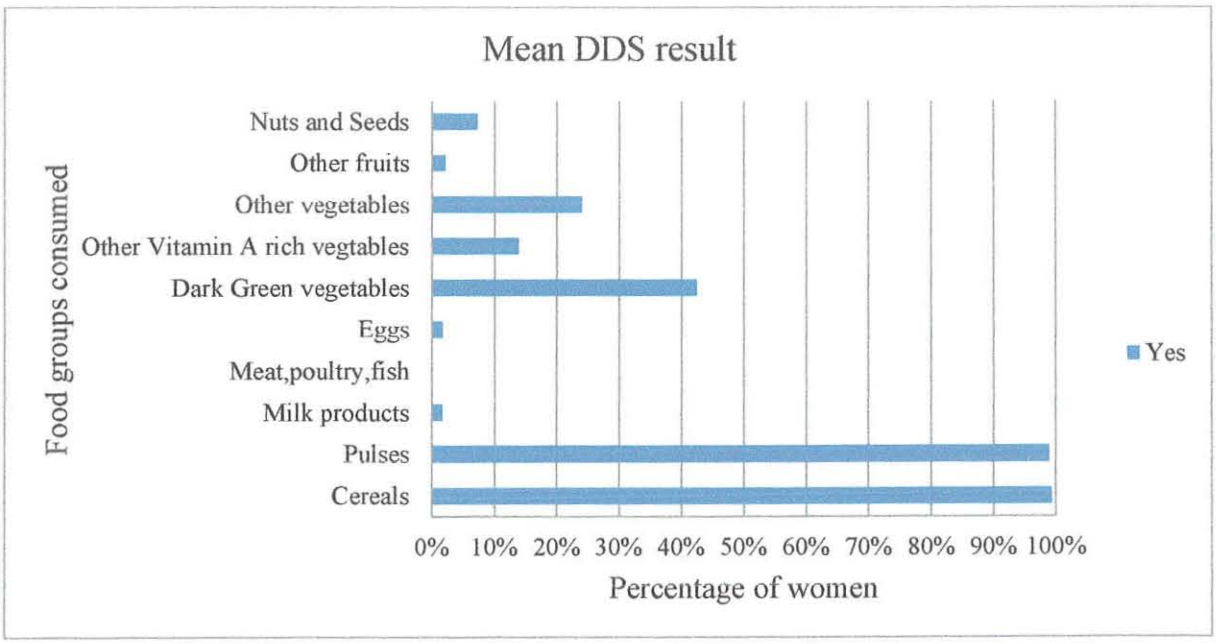

Figure 4. 3 Mean dietary diversity score results

4.3. Women's Dietary diversity

Nearly half of anemic (54%)and non- anemic (53%)women consumed three food groups while nearly one third of anemic (30%) and 21% of non-anemic women were consumed only two food groups, these are known to be cereals and pulses that formed the integral part of the main meal. Furthermore, (14%) of anemic and 22% of non-anemic women were consumed four food groups, while less than five percent of anemic (2%) and non-anemic (4%) women have consumed five food groups. Neither of women has consumed more than five food groups. Starchy stables, pulses, dark green vegetables and other vegetables were the most frequently consumed products. Other fruits, animal products were least consumed the food groups (Figure 4.4).

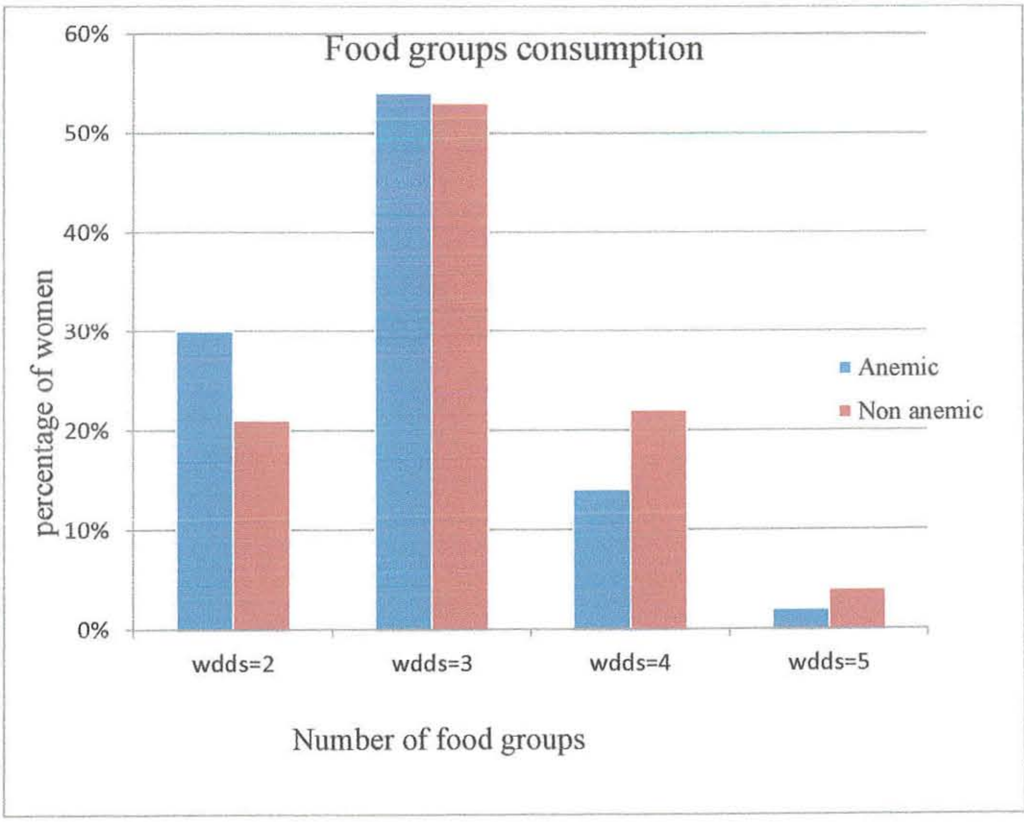

Figure 4. 4Dietary diversity score of anemic and non -anemic women

4.4. Food consumption based on food frequency questionnaires results

Almost all study participants were consumed cereals (100%) and pulses (99%) two times and above per week. Cereals (maize, sorghum, millet, teff) and pulses (lentil, chickpeas) were commonly consumed foods in study area. Close to half (45%) of study participants consumed dark green vegetables, other vegetables from at least once weekly to once daily. One-fourth (25%) of participants consumed other vitamin A rich vegetables below two times weekly. Consumption of nuts and seeds, other fruits and animal products was rare or negligible (Figure 4.5).

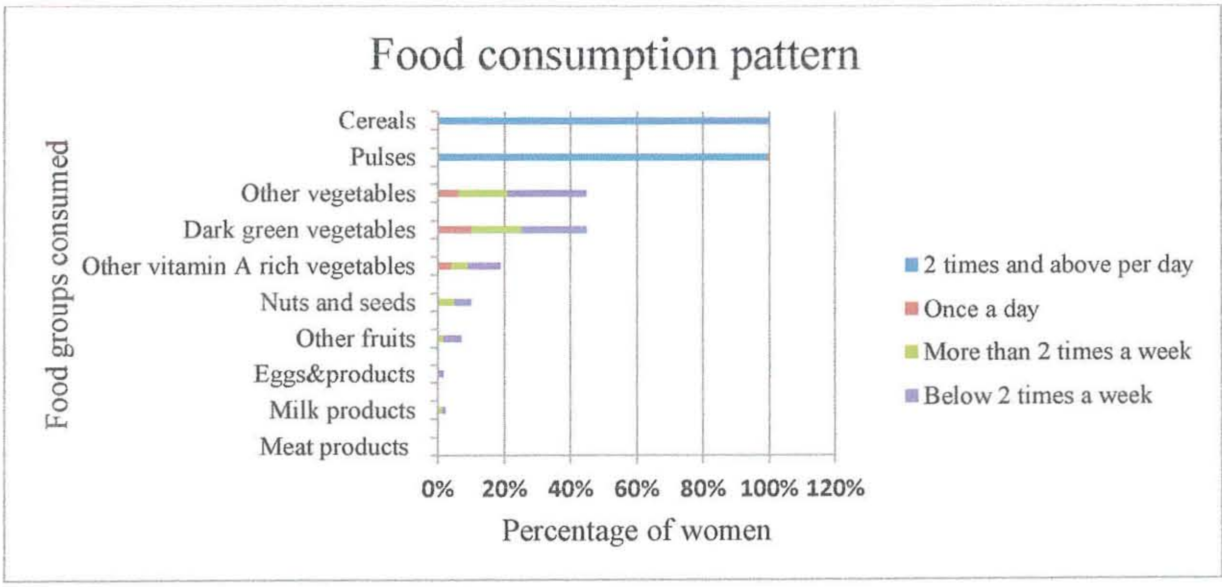

Figure 4. 5 Seven-day food frequency consumption result

4.5. Dietary diversity score results based on FFQ

Low DDS was shown in , where the main score, based on a seven day recall for 15–49 year old women, was (3.5 ±0.92) out of 10 food groups for all participants. From all study participants nearly (10%) of anemic and (7%) of non-anemic women were consumed only two food groups. Half of anemic (50%) and 41% percentage of non-anemic women were consumed only three food groups. One-fourth of anemic (26%) and one-third (36.8%) of non-anemic women were consumed four food groups. Close to 10%, anemic and non-anemic women were consumed five food groups. Insignificant number of anemic (2.4%) and non-anemic (4.2%) women was consumed six food groups. Number food groups consumed in this case range from one to six (Figure 4.6).

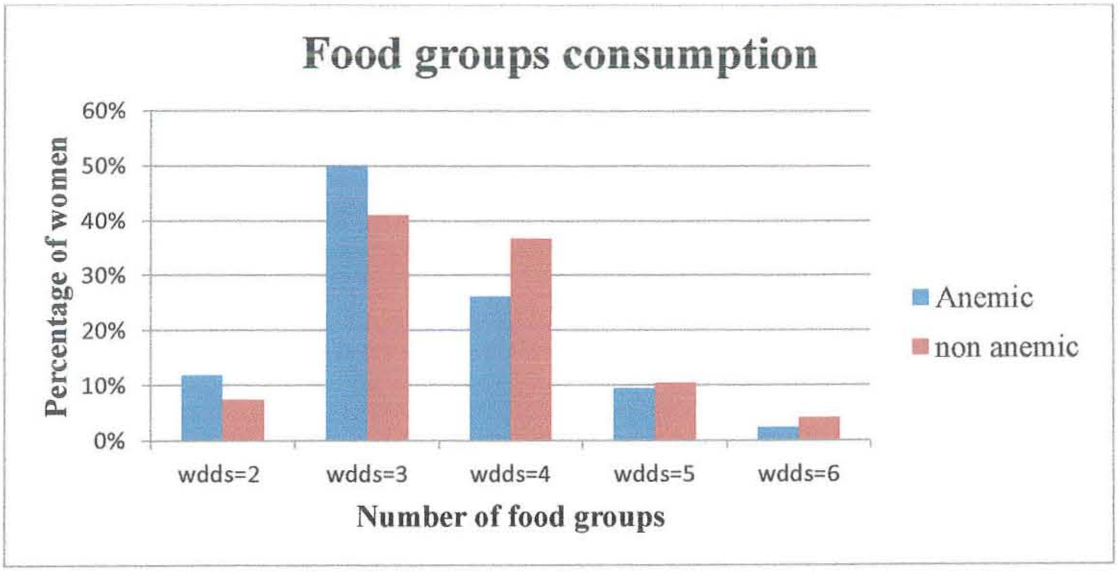

Figure 4. 6 Seven day dietary diversity score

4.6. Minimum dietary diversity women (MDD-W)

Only six women out of 179 consumed five or more of the ten specified food groups the previous day. Relatively the mean MDD-W for the anemic women (2.89±0.728) was generally low compared to nonanemic women (3.09± 0.773). Over all, the mean MDD-W for the sample was 3.00± 0.757. The percentage of WRA who achieved minimum dietary diversity (≥ 5 food groups) was only 3%, and they are more likely to have higher (more adequate) micronutrient intakes than those of 97% of women who did not meet the minimum dietary diversity (Table 4.3).

Table 4. 3Minimum Dietary diversity women (MDD-W).

Total	MDD-W (Mean ±SD)	Status	MDD-W (Mean ±SD)	Percentage of women	
				<5 Food Groups	≥5 Food Groups
(N=179)	3.0 0±.757	Anemic (N=84)	2.89±0.728	98	2
		Non anemic(n=95)	3.09±0.773	96	4

4.7. Prevalence of iron deficiency anemia

Among all anemic women (84), (26.2%) were iron deficient (serum ferritin < 30 µg/L), this indicates iron deficiency as a moderate public health problem in the study area. Furthermore, close to 8% WRA, had tissue iron deficiency based on soluble transferin receptor (sTfR) level above 8.3mg/L. Nearly 6% of them had low body iron store (BIS), ratio of serum ferritin and serum transferin receptor. From all non-anemic women (95), (10.5%) of them, had low iron stores based on serum ferritin levels below 30µg/L. Only 1% of them, had tissue iron deficiency based on sTfR levels above 8.3mg/L. only 2% of them had low BIS level below 0mg /kg. Over all, the mean prevalence of iron deficiency anemia measured with serum ferritin was 18.4% (95% CI: 10.5%-26.2%). According to this result, anemic women have low serum ferritin level than non-anemic women (Figure 4.7).

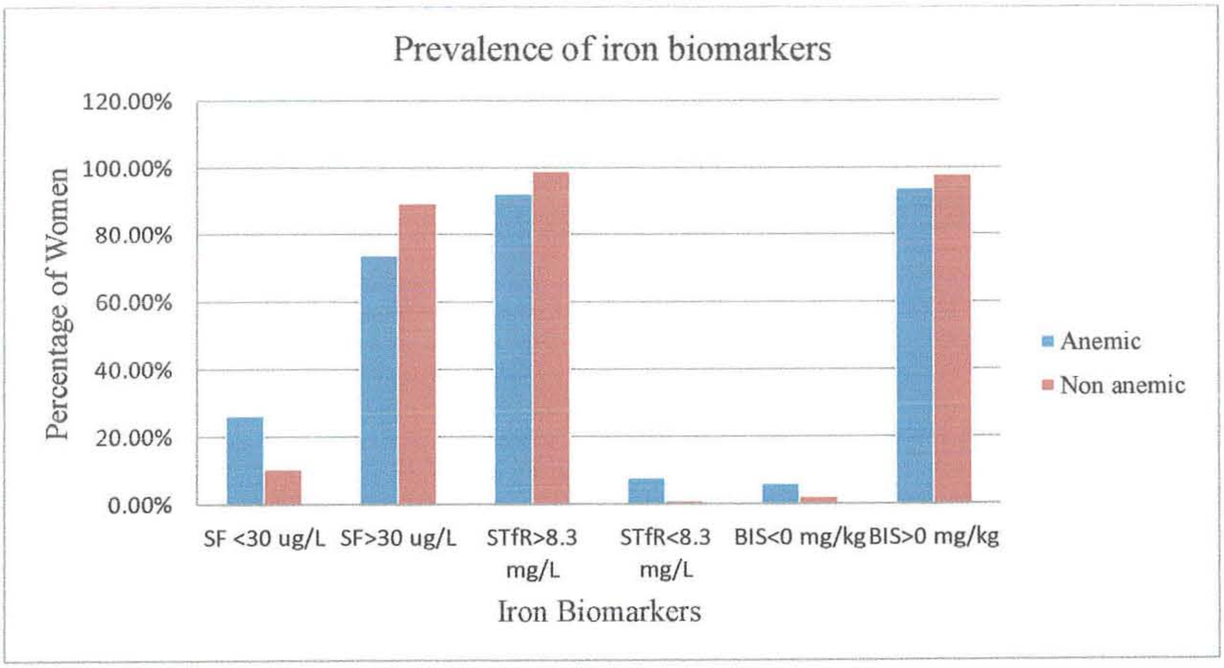

Figure 4. 7 Prevalence of iron biomarkers in anemic and non- anemic women. SF, serum ferritin; sTfR, serum transferrin receptors

4.8. Prevalence of vitamin A and Zinc Deficiency

About 72.6% of anemic and 60% of non-anemic women were zinc deficient (serum zinc < 10.7 μmol/L (70 μg/L) (Figure 4.8).Furthermore, 13 % of anemic and 12 % of non-anemic women were vitamin A deficient (RBP < 0.81 μmol/L). To sum this up, 65 % and 12.4 % of women (both groups) were zinc and vitamin A deficient.

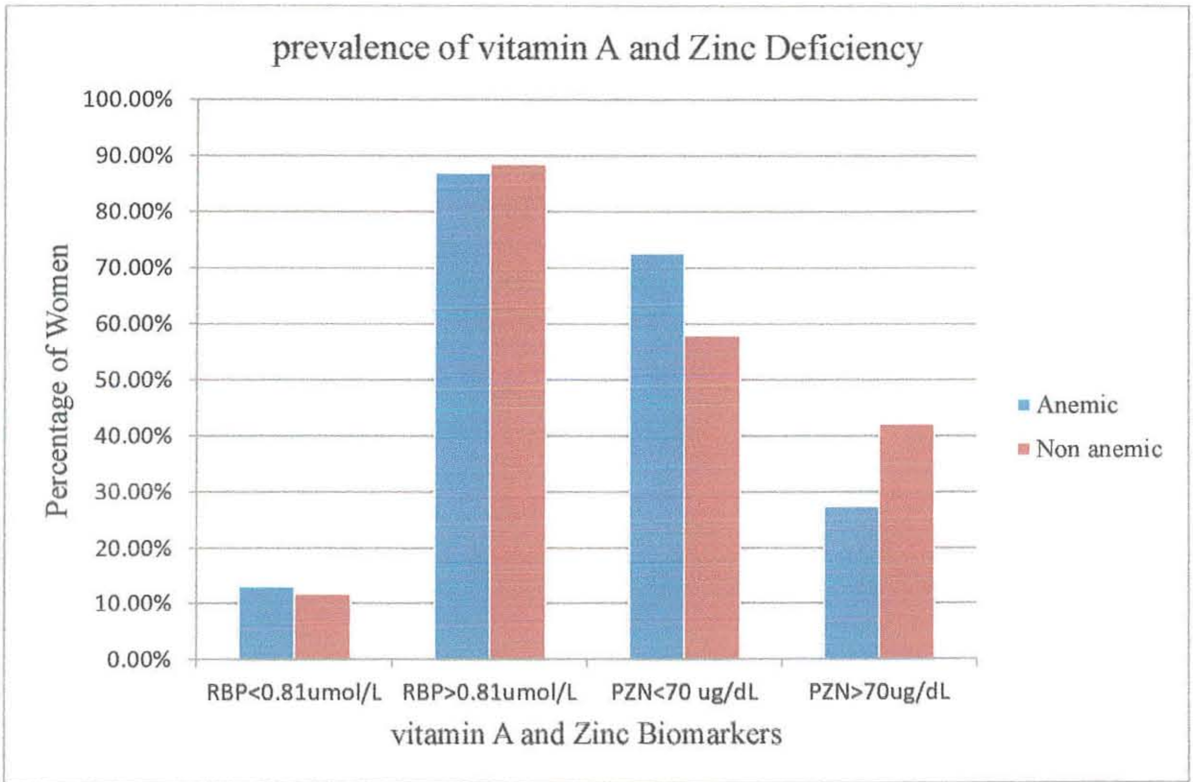

Figure 4. 8Vitamin A and zinc deficiency status in anemic and non-anemic women.RBP, retinol-binding protein; PZN, plasma zinc

4.9 Prevalence of inflammation status in anemic and non anemic women

Less than 5% of anemic women were in late convalescence phase (CRP<5mg/L and AGP>1mg/L), 1.2% were in early convalescence phase (CRP >5mg/L and AGP >1gm/L), and 3.6% in incubation phase (CRP>5mg/L and AGP<1gm/L). No inflammation/infection observed in 90% of the subjects. Furthermore, 4.2 %, 2.1 % and 5.3 % of non-anemic women were in late (CRP<5mg/L and AGP>1mg/L) and early convalescence (CRP >5mg/L and AGP >1gm/L) and incubation phase (CRP>5mg/L and AGP<1gm/L), respectively (Figure 4.9).No infection/inflammation was observed in 88.4 % of the women in this group.

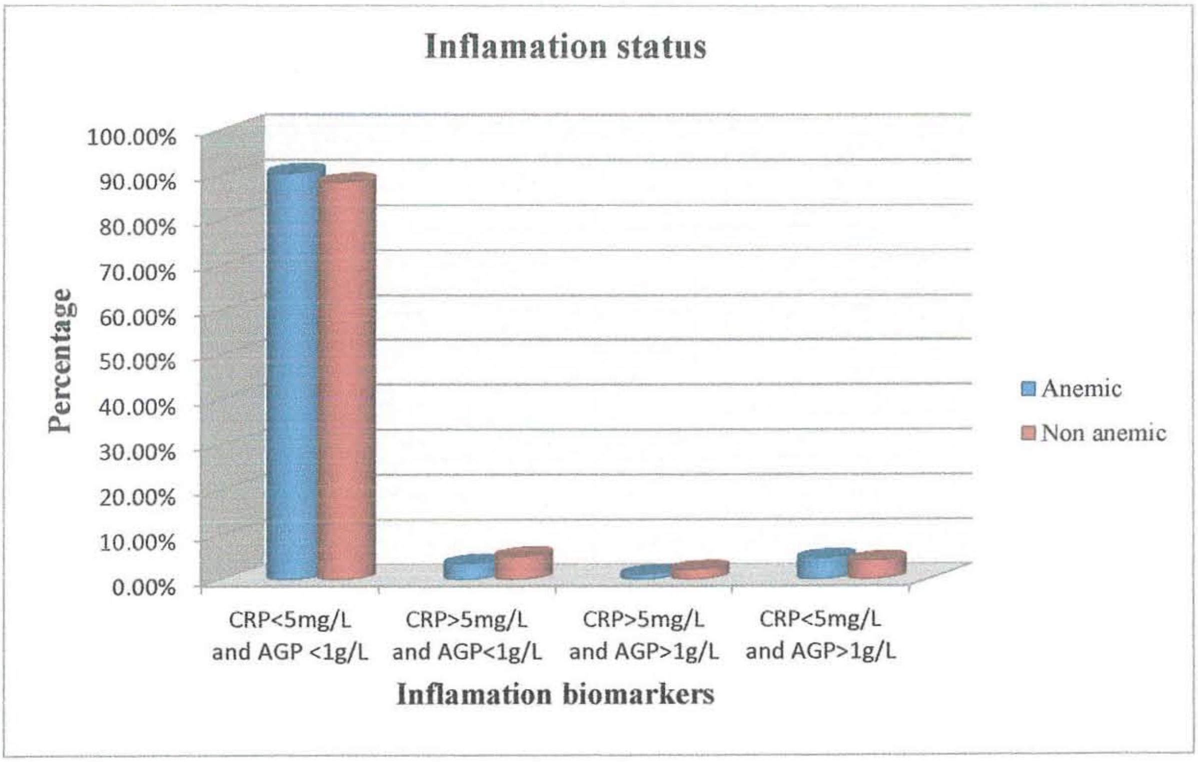

Figure 4. 9Inflammation status of anemic and non-anemic women.CRP, C - reactive protein; AGP, Alpha- 1-acid glycoprotein

4.10. Association results between dietary diversity score and nutritional status

The present study revealed positive association between dietary diversity, and hemoglobin level, serum ferritin, body iron store, retinol binding protein and serum zinc (Table 4.4). DDS (m=3.0 ±0.757) and serum ferritin (m=69.6±42.85),r(179)=0.913, p<0.01,serum zinc(m=62±25.4),r(179) =0.295,p<0.01,bodyironstore(m=7.3±3.2),r(179)=0.764,p<0.01,RBP(m=1.43±0.76),r(179)=0.24 2,p<0.01,Hb (m=12.23±1.43),r(179)=0.235,p<0.01.in contrary to the result described before there was negative association(inverse correlation) between dietary diversity score and soluble transferrin receptor (sTfR) (m=4.94±1.5), r (179) =-0.202, p<0.01.The result also showed an inverse correlation between serum ferritin and sTfR, indicating the concentration of sTfR increase in iron deficiency and IDA [125]. In all, selected biomarkers had statistically significant association with dietary diversity.

Table 4. 4 Association between dietary diversity score and nutritional status

	Dietary diversity score	
	Pearson correlation	**Sig.(2-tailed) p value**
Serum ferritin	0.913	0.000**
Hemoglobin level	0.235	0.002**
Serum transferin receptors	-0.202	0.007**
Body Iron Store	0.764	0.000**
Retinol Binding protein	0.242	0.001**
Serum zinc	0.295	0.000**

5. DISCUSSION

We investigated the dietary diversity pattern and micronutrient status as well the predictive ability of dietary diversity on WRA's micronutrient status. According to the study diets of women of reproductive age were dominated by cereals (99.4%) predominantly; maize, millet, sorghum, teff, and pulses. They provide energy, varying amounts of micronutrients (e.g., certain B vitamins provided by grains), and contains varying amounts of anti-nutritional factors. Animal products, fruits and vitamin A rich vegetables which are good source of macro and micronutrients were hardly taken by the women and far below recommendations[126]. Rare consumption of animal products in study area may be seems related to fasting period prescribed by Ethiopian orthodox church that occurred in early November and December. However, several studies also revealed the limited intake of animal products despite the fasting season and existence of a large population of livestock, especially in rural areas[67]. Furthermore, low consumption of dark green vegetables, other vitamin A rich vegetables, fruits, vegetables might be due to limited food supply and nutritional knowledge, unbalanced dietary practices that are also characteristics of less developed areas [55, 127]. This result shows that WRA in Bahir Dar zuria and Dangla sub-districts mainly depend on monotonous diet, based mainly on energy dense, but micronutrient poor starchy staples which are common source of foods in food insecure areas and have highly contributed to the burden of malnutrition, particularly, inadequate micronutrient intake [5, 20, 69].

Dietary diversity is a key measure of sustainable diets and is currently being considered as one of the principal indicators in the post-2015 Sustainable Development Goals with key objective of improving the diets of smallholder families in terms of both quantity and quality [90, 128]. Mostly, monotonous staple diets lack essential micronutrients which lead to micronutrient deficiencies, particularly in WRA[129]. In this study dietary diversity which was measured by using a simple score over a 24 hour period [27],this showed that nearly 79% of WRA consumed either two to three food groups, one-fifth(18%)of women consumed four food groups, and 3% women consumed five food groups. Over all, this finding indicated that WRA who met minimum dietary diversity were negligible (3%). This result was lower than some studies conducted in Nigeria, South Africa, Kenya and Algeria[4, 5, 26, 130, 131] and others such as done by Patricia, et al (2016) (<15%), Gitagia et al (2019) (19%),Chakona& Shackleton(2017)(25%)[5, 26, 36]. The result was lower than previous findings that reported DDS of 5.81, 3.78, 3.81, 3.8, and 4.2 in Nigeria, South Africa, Kenya, Algeria, and Kenya respectively[128].

The low DDS of the rural women in this study is, however, similar to that carried out on rural women in Nigeria Abia state that reported a mean DDS about three [36]. This result is comparing well to the study conducted in South Africa with similar DDS score[132]. The finding also agrees with those of other studies that most diet in developing countries is predominantly cereal based[20]. Consumption of low quality, cereal based diet may be due to lack knowledge of women about importance of diversified diet for health, extreme poverty, and backward agricultural practice. The findings agree with those of other studies that most diet in developing countries is predominantly cereal based [23, 76, 133]. Notably, diets in developing countries have also been reported to be lacking or having little animal source foods [65, 134].

According to this information rural women of the study area mainly consumed monotonous diets which are of low quality ,cereal based diets thereby increasing the risk of micronutrient deficiencies which is already high in some other resource poor countries in addition to study area[130] and acquiring all micronutrients from one or two food groups is not plausible and requires regular intake of several food groups in sufficient quantity and variety to satisfy the nutritional needs[20, 40]. In the present study, these women cannot afford to diversify their diets with adequate amounts of fruits, vegetables or animal-source foods that contain different essential micronutrients, deficiencies are inevitable.

The MDD-W tool has been used as an indicator of access to food and was able to distinguish women with different levels of vulnerability to micronutrient deficiency[124]. Women who consumed food items from ≥5 of the ten food groups were considered more likely to consume foods from animal sources, pulses, nuts or seeds, and fruits or vegetables and to have a greater probability of micronutrient adequacy than those who consume <5 food groups[135]. There was no significant difference in MDD threshold between anemic and non-anemic women based on our findings. When using a mean MDD-W of five or less food groups to define a poor dietary intake and food insecurity, results revealed that 97% of women in the study sites had failed to achieve the minimum dietary diversity, and are hence more likely to have inadequate micronutrient intake which increases their vulnerability to food insecurity. Outcome of the study was within the range reported in other South African studies where very low DDS observed [5], although there are no reports on women of reproductive age.[70, 90]

Different studies showed that consumption of cereal based diet could lead to iron deficiency as the iron present in this food items is less absorbable[105].Therefore,18.4% of iron deficiency in the area might also conform this findings. The level of iron deficiency found in this study is also in agreement to the result of first nationwide survey conducted in Ethiopia in 2005 (17%) [32],and other systematic analysis of national surveys in SSA and south east Asia in 2015 (16%)[85]. Still, our finding lower than studies in Mozambique (27.4%)[59] and higher than studies in Nigeria(9.7%)[136].The low prevalence may be due to high consumption of oil extracted from palm fruit that is rich in precursors of vitamin A, which have positive influence on body iron status. The low prevalence may be due to high consumption of oil extracted from palm fruit that is rich in precursors of vitamin A, which have positive influence on body iron status.

Furthermore, the prevalence of zinc deficiency in women of reproductive age in study area was 65%[137]which was higher than reported in democratic republic of Congo (52%) [138] and cross-sectional study conducted in Mozambique(32.7%) [39]Similarly the prevalence of low SZC in women of reproductive age in study area was comparable with other research conducted in Vietnam (67%), and Cambodia (63%) but it was less than Cameron that recorded higher SZC (83%)[137].The higher prevalence of zinc deficiency in study area may be due to the daily consumption of cereals and legumes in which zinc absorption is low due to the presence of anti-nutritional factors such as phytate[139]. Phytate are present in plant products, particularly grains and legumes, and form insoluble complexes that limit zinc absorption [48]. They are of particular importance in developing countries, where largely plant-based, low-animal protein diets are common [59].

According to our study prevalence of vitamin A deficiency, which was measured with retinol binding protein[140, 141]was 12.4%[62]. A cross-sectional study conducted in Mozambique found comparable result (14.7%) [39]. This result seems low when compared with study conducted in Cameron 2009 on women of reproductive age that inflammation adjusted prevalence was 21.9%(<1.17μmol/L)[140]. But other studies conducted in Nigeria[136], Cameron[142],Côte d'Ivoire[143]reveals that vitamin A deficiency seems virtually none exist in women of reproductive age though different cut-off points implemented. This shows that these countries are using their effort to minimize burden of vitamin A deficiency through different mechanisms such as fortification and biofortification programs.

Good nutrition is fundamental for good health. Maternal nutrition in particular represents a major public health challenge because it affects not only women's health, but also that of future generations. The main finding of this study was that women in study area were not only failed to consume diversified diet but also they have poor micronutrient status. Nutritional status is considered an outcome of biological processes that involve food utilization while dietary diversity is a measure of diet quality and is quantified as food variety (the number of individual food item types consumed) or food group variety (the number of food groups utilized)[132].

This study also revealed the positive association between dietary diversity score and serum ferritin, body iron store, retinol-binding proteins, hemoglobin level and zinc status. However, negative association observed between DDS and serum transferrin receptors. This shows DDS as a significant predictor of vitamin A, iron deficiency anemia, and zinc insufficiency while controlling the co-variants. This means that women who had a greater dietary diversity achieved an improved intake and/or absorption of vitamin A, zinc and iron in the blood.

According to Kiboi, Kimiywe [20] a positive linear relationship between dietary diversity and the women's anemia status was observed. Other cross-sectional study conducted in Ghana also finds strong correlation between hemoglobin concentration and DDS[144]. Study conducted in Mozambique found that low DDS was associated with higher odds of having low serum zinc compared to having a higher score [68], Furthermore, studies in Ethiopia also found an association between DDS and serum zinc among pregnant women [55]. Cross-sectional study conducted in Northern Kenya found that DDS had a significant positive effect on serum retinol concentration[145]. Indeed, previous studies in pregnant women in Southern Ethiopia and breastfeeding women in Northern Kenya have found that women with a low DDS had an increased risk of having vitamin A deficiency compared to women with a higher DDS [55].

In the present study, positive correlations were noted between dietary diversity score and all the selected micronutrients. This suggests that increase in nutrient intake through increased dietary diversification could lead to a better micronutrient status. These findings are consistent with those of studies done by Chakona et al.,(2017)[5]and torheim et al.(2010)[73]. The negative correlation between high level of dietary diversity score and lower level of serum transferin receptor (sTfR)suggested that higher dietary diversity score ,related to lower level of sTfR, which mainly indicate tissue iron deficiency returned to normal value [146]. The finding of the present study was also in agreement with Turgeon et al.(2016)[108].

6. LIMITATION OF THE STUDY

Even though one of the exclusion criteria in this study was pregnancy, confirmation was done simply by asking women whether they were pregnant or not rather than laboratory test for determination.

7. CONCLUSION AND RECOMMENDATION

The main finding of this study was that women in study area were not only failed to diversify their diet mainly consume starchy staples and pulses but also they have poor micronutrient status. The daily menu for women in study area was mostly **injera** (እንጀራ) or bread made from sorghum, maize, finger millet and teff and **kik/shiro wot (ክክ /ሽሮ ወጥ)** made from chickpea, peas. As indicated by our study 97% (both groups), of WRA in the study area failed to fulfill (MDD) with very low mean dietary diversity score (3.0±0.757) made it difficult for MDD-W to predict quality of women's diet. Meanwhile laboratory finding indicates the prevalence of iron deficiency anemia, zinc deficiencies in WRA in study area are a public health concern based on the WHO criteria. Furthermore, this study showed a favorable association between dietary diversity score and the quality of diet, indicating low dietary diversity was associated with higher odds of having low serum zinc and iron biomarkers. Low dietary intake, poor nutrition knowledge, economic problems and traditional farming that harvest the same type of crops year after year may be the most important problems for their dependent on monotonous diets.

Therefore, stakeholders have to develop nutrition sensitive programs that should focus on improving micronutrient intake through stimulating dietary diversity and the fortification of commonly consumed and affordable food products like sugar, flour. In addition to these modern technologies, supporting agricultural practice like on-farm diversity is an important benefit to improving dietary diversity and quality and provides a potential income generation mechanism to sell and buy those foods in local markets to make their food of higher quality. In addition, improvement of the transport system to give access to locals to sell their local produce to raise incomes for their families is important.

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ANNEXES

Annex 1: Ethical Clearance from college of natural and computational science, Addis Ababa University

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

Ref. No.
CNSDO/268/10/2018
Date
January 30, 2018

To Whom It May Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 11/01/2018 Minute No. IRB/031/2018 has examined the project proposal entitled "**Association between dietary diversity score, anemia, iron and zinc Biomarkers among women of reproductive age in west Gojam zone, Amhara Region, Ethiopia**" by Tadiwos Hassen.

The proposal is approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean, College of Natural & Computational Science
Addis Ababa University
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Please Quote our reference number in you correspondence

"Examine all things hold fast that which is good"

Annex 2: Participant's Information Sheet and Consent Form

English version information sheet and consent form for participating in the study, "Association between dietary diversity score, anemia, iron, zinc and vitamin A biomarkers among women of reproductive age in west Gojam and Agew/Awi zone", Amhara Region, Ethiopia.

Identification Code: -------------------------------------

Hello, how are you?

My name is TadiwosHassen; I am a Masters Student in AAU, college of natural sciences, in food science and nutrition center as a principal investigator conducting this study.

The objective of this study is to assess the association between dietary diversity score, anemia, iron, and zinc and vitamin A biomarkers in women of reproductive age in west Gojam&Agew/Awi zone specifically to investigate the association between dietary diversity score and anemia, and micronutrient status of women of reproductive age in these rural areas.

Your cooperation and willingness to participate in the interview& providing blood sample is very helpful in identifying the food consumption pattern and most missing micronutrient in this area.

I assure you that all information that you give will be kept strictly confidential and all information and blood sample taken from you used only for this research purpose. Your participation is voluntary and you are not obliged to answer any question you do not want to answer. If you are not still comfortable with interview, please be free to stop me any time you like there is no harm if you don't answer the questions and no special benefit you get except knowing your micronutrient status at the end of the study.

If you are willing to participate, the interview questions and measurements will take 20-30 minutes. I would like to interview you few questions regarding socio-demographic characteristics; your food consumption pattern in the last 7 days and last 24 hours and blood samples will be collected at once after you finished your questionnaires

We would be thankful if you spend some time with us answering questions.

1. If yes, Name of interviewer_________ Signature________

2. if not, skip to the other participant

For more information and question here is the contact address of investigator:

TadiwosHassen: mobile: 0913286553; e-mail: tadiohassen@gmail.com.

Annex 3: Amharic participant's consent and information sheet

<u>**በጥናትና ምርምር ላይ ለመሳተፍ ፍቃደኝነትን መጠየቂያ ቅጽ፤**</u>

እንደምን ነሽ?

እኔ ታዲወስ ሀሰን እባላልሁ፤

በአድስ አበባ ዩኒቨርስቲ የተፈጥሮ ሳይንስ ኮሌጅ ዉስጥ ለማደርገዉ የማስተርስ ዲግሪ የማሟያ የጥናትና የምርምር ስራ እየሰራሁነዉ::

በወሊድእድሜላይ የሚገኙ ሴቶች አመጋገብና በሰዉነታቸዉ ወስጥ ያሉ ንጥረነገሮችን ግንኙነት በተመለከተ እና ያላቸዉን አስተዋፅኦ ለማጥናት በዚህ ጥናት ተሳታፊ እንዲሆኑ የእርሶን ፍቃደኝነት በአክብሮት እየጠየቅሁ፤የእርሶ ፍቃደኝነትና በጎ ትብብር ጥናቱ ለህብረተሰባችን አመጋገብ ስርዓት መሻሻል እና በሰዉነታቸዉ ዉስጥ በቂ ነጥረ ነገሮች መኖራቸዉን ለመመርመር እና ክትትል ለማድረግ እንደግብአት ትልቅ ጠቀሜታ ይኖረዋል ::

በመሆኑም እርስዎ በምርምር ስራው ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ፤ የሥነ - ምግብ ባለሙያው ለሚጠይቆት ጥያቄ ጊዜ ወስደዉ የበኩልዎን መረጃ በመስጠት፤ እና የደም ናሙና በመስጠት እገዛ እንዲያደርጉ በአክብሮት እጠይቃለሁ::

የሚሰጡት ምላሽ እና የደም ናሙና ከጥናትና ምርምር ስራው ውጪ ለሌላ አገልግሎት አይውልም:: በመጠይቁ ላይ ከተመለከቱት ጥያቄዎች መካከል መመለስ የማይፈልጉት ጥያቄ ካለ አለመመለስ ይችላሉ:: በጥናቱ ላይ በመሳተፍዎ የራስዎን የግል የጤና ሁኔታ በጥናቱ መጨረሻ ላይ ከማወቅ ውጪ የተለየ ጥቅም አያገኙም።

ከላይ በተገለጸውን ቅጽ መስማማትዎን ፊርማዎ ያረጋግጡ ________________

መረጃው የተሰጠበት ቀን ________________________________

የአጥኚው ፊርማ ____________________________________

Annex 4: Questionnaire (English Version)

Individual's ID: __ __ __ ___

Date of Interview: __ __/ __ __/ 2018

Time started: __ __: __ __ AM/ PM Time Ended: __ __: __ __ AM / PM

Name: ________________ __________

Physical Address: __________, Wereda: __________ Kebele: ________ H.No:____

Role of participant in the study: Anemic and non anemic /Control

Methods of Assessment is classified in to three: I. screening for anemia

II. Questionnaires

III. Phlebotomist will take blood and

IV. Biochemical Analysis

I.SOCIO-DEMOGRAPHIC QUESTIONS

No	Questions	Response	Code
1	Age of women in complete years	--------- years	
2	Educational level	1. No formal Education ☐ 2. Primary Level ☐ 3. Secondary Level ☐ 4. Tertiary Level ☐	
3	Marital status	1.Single ☐ 2.Married ☐ 3.divorced ☐ 4.widowed☐ 5.separated ☐	
4	Occupation	1.House wife ☐ 4.civil servant ☐ 2.Trader ☐ 5.other (specify) ☐ 3.Farmer ☐	
5	Monthly Income earned	1. None ☐ 4.2000-3000 ☐ 2. Below 1000 ☐ 5.≥3000 ☐ 3. 1000-2000 ☐	
6	Number of children	1. None☐2. 1-3 ☐3.4-6 ☐ 4.>6☐	
7	Number of children in different age category	1.0-2 years ☐ 2. 3-5 years ☐ 3.> 6 years☐	
8	House hold size	1.1-3 2.4-6 3.> 6	
9	Do you take any medication?	1.Yes ☐No ☐	
10	If you say yes to Q. 9 what medication do you take?	1.contraceptive ☐2.other medicine ☐	
11	Do you experience abortion in recent months	1. Yes☐ 2. No ☐	

II.FOOD FREQUENCY QUESTIONNAIRES;

How often, in the past 7days did you eat the following?

List of foods	Range of frequencies				
	Never	Weekly		Daily	
	Never	<2x/wk	3-6x/week	Once/day	>2x day
Starchy staples (rice, wheat, sorghum, millet, teff, maize, oats, barley, finger millet,cassava,potatoes etc. or foods made from them; injera, bread, porridge, dry roasted or boiled grain, pasta/macaroni)					
Beans and peas (chickpeas, Lentils, peas, beans, broad beans, soya and soya products)					
Nuts and seeds (sesame, Nuts, seeds, sun flower and products made from these (stew, groundnut, peanut butter, etc.)					
Milk & Milk products (cow milk, powder milk, yogurt, cheese, whey)					
Flesh foods(chicken, beef, goat, sheep, fish or meat products sausages, stew, fresh, dried & canned like roasted, stew, lebeleb, red meat ,processed meat etc)					
Eggs (fried, boiled, omelet in salad, in baked goods, etc.)					
Vitamin A rich dark green leafy Vegetables (fresh & canned; cabbage, kale, spinach, broccoli, dark green lettuce, mustard green , collard green ,green pepper etc.)					
Other vitamin A rich fruits and vegetables(orange fleshed sweet potato, ripe mango ,ripe papaya ,carrot, melon orange fleshed banana orange fleshed squash ,red palm fruit, watermelon, pumpkin)or juice made from them					
Other vegetables(tomato, cucumber, okra ,legumes with green pods etc)					
Other Fruit (fresh & canned; whit bananas, oranges, flesh coconut , avocado, unripe mango, unripe papaya guava, peach, pineapple, apples, tringo . or juices made from them)					

III. WATER SOURCE

1. What is the main source of drinking water for members of your household?
   A) Pipe water

   B) Water from spring

   C) Surface water (river, lake, dam, stream etc)

   D) Rain water

   E) Other

2. How long does it take to go there, get drinking water, and come back? (Not include waiting time) In minute ---------------------------
   1. Walking --------------
   2. Bajaj---------------
   3. Carts ---------------

3. Do you do anything to the water to make it safer to drink?
   No----------------------------------
   Yes ---------------------------------
   Don't know ---------------------------

4. If so what do you do to make the water safer to drink? Anything else?

   A. Boil
   B. Water purifying product/ water guard/ bishangari / aquatabs/
   C. Other bleach /chlorine/waha agar
   D. Strain through a cloth
   E. Ceramic filter
   F. Let it stands and settles
   G. Other *(specify)*
   H. Don't know ....

IV.DIETARY DIVERSITYASSESSMENT

1. Have you eat foods made from starch staples (cereals and root tubers) like injera, bread, porridge, nifro,kolo) that made from teff, wheat, barley, millet, sorghum, maize etc in last 24 hours? 1. Yes 2.No
2. Have you eat foods made from legumes and pulse (kikwot, shirowet,nifro, kolo) and other foods made from them in last 24 hours? 1.yes 2.No
3. Have you eat/drink any milk products (yogurt, cheese, raw milk, and powder milk) in last 24 hours? 1. Yes 2.No
4. Have you eat any meat products from (fish, poultry, flesh meat, goat, and sheep) in the form of tibs, kikil, keywet, raw meat and other foods in last 24 hours?
   1. Yes 2.No
5. Have you eat any egg products (firfir, enkulal sills, and kikil) and other foods made from them in last 24 hours? 1.yes 2.No
6. Have you eat vitamin A rich green vegetables like cabbage, green peppers, dark green lettuce, spinach, green pepper, kale, broccoli etc and any food made from them in the last 24 hours? 1.Yes 2.No
7. Have you eat other vitamin A rich fruits and vegetables like ripe mango, ripe papaya carrot, orange fleshed sweet potato, water melon, orange fleshed banana, palm fruits, apricot and juices made from these vegetable and fruits etc in the last 24 hours? 1. Yes 2.No
8. Have you eat foods made from nuts and seeds like sesame, ground nut, sun flower, peanut and other oil seeds in the last 24 hours? 1.yes 2.No
9. Have you eat other vegetable products like onion, tomato, cucumber etc in last 24 hours? 1.yes 2.No
10. Have you eat other fruits orange, white banana, unripe mango, unripe papaya, avocado, guava, pineapple, apple, mandarin, lemon, flesh coconut etc and juices made from them in the last 24 hours? 1.yes 2.No

Annex 5: Questionnaires (Amharic version)

ቃለመጠይቅ

የግለሰቧመለያኮድ______________________________

ቃለመጠይቅየተካሄደበትቀን______________________________

ቃለመጠይቅየተጀመረበትሰዓት____________ያበቃበትሰዓት____________

ስም______________________________

የመኖሪያአድራሻ______ቀበሌ______ወረዳ______ዞን______

የተሳታፊዎችሚናየደምማነስያለባትየሌለባት ☐ ☐

1.የግለሰቧ ማህበራዊስነህዝባዊእናኢኮኖሚያዊሁኔታመጠይቅ

ተ.ቁ	ጥያቄ	መልስ	ኮድ
1	የተጠያቂዋእድሜ	______ዓመት	
2	የተጠያቂዋየትምህርትደረጃ	1. መደበኛ ት/ት ያልተማረች 2. መጀመሪያደረጃ 3. ሁለተኛደረጃ 4. ከሁለተኛደረጃበላይ	☐ ☐ ☐ ☐
3	የጋብቻሁኔታ	1. ያላገባች 2. ያገባች 3. የተፋታች 4. የትዳርአጋሯንበሞትያጣች 5. ለጊዜውየተለያየች	☐ ☐ ☐ ☐ ☐
4	የስራሁኔታ	1. የቤትእቤት 2. ግብርናላይየተሰማራች 3. ነጋዴ 4. የመንግስትሰራተኛ 5. ሌላካለይጥቀሱ	☐ ☐ ☐ ☐ ☐
5	የተጠያቂዋየወርገቢ	1. መደበኛየገቢምንጭየሌላት 2. ከ1000 ብርበታች	☐ ☐

		3. ከ1000-2000 4. 3000 ብርበላይ	
6	የልጆችብዛት	1. የለም 2. 1-3 3. 4-6 4. ከ6 በላይ	
7	የልጆችየእድሜደረጃ	1. ከ0-2 ዓመት 2. ከ3-5 ዓመት 3. ከ+6 ዓመትበላይ	
8	ጠቅላላየቤሰብብዛት	1. 1-3 2. 4-6 3. 6 በላይ	
9	በአሁኑሰዓትየምትወስጂውመድሃኒትአለ	1. አዎ 2. አይደለም	
10	የዘጠነኛውመልስአዎከሆነከሚከተሉትየትኛውን	1. የወሊድመቆጣጠሪያ 2. ሌላመድሃኒት	
11	ባለፉትጥቂትወራትውርጃአጋጥሞሽያውቃል	1. አዎ 2. አላጋጠመኝም	

2.ከዚህበታችየተዘረዘሩትምግቦችምንያክልጊዜእንደተመገቡየሚዳስስመጠይቅ

ባለፉትሰባትቀናትከዚህበታችየተዘረዘሩትንምግቦችምንያህልጊዜተመግበዋል፡፡

የምግቦች ዝርዝር በየፈርጁ የተዋቀረ	የድግግሞሽ ደረጃ				
	በፍጹም	በሳምንት		በቀን	
		ከ2ጊዜ በታች	ከ3-6ጊዜ	በቀን 1ጊዜ	በቀን 2 እና ከ2 ጊዜ በላይ
የአገዳና የብእር እህሎች (ሩዝ፣ስንዴ፣ገብስ፣ማሽላ፣በቆሎ፣አጃ፣ጤፍ፣ዳጉሳ እና ከነዚህ የሰብል አይቶች የተሰሩ እንጀራ ዳቦ፣ቂጣ፣ንፍሮ፣ፓስታ፣መኮረኒ፣የመሳሰሉት					
የጥራጥሬ አይቶች አተር፣ባቄላ፣ምስር፣ሽንብራ፣አኩሪአተር፣አደንጓሬ፣ጓያ ወይም ከነዚህ ጥራጥሬዎች የተሰሩ ክክ ወ፣ሽሮ ወጥ፣ቆሎ፣ንፍሮ፣ስልጆ፣ፉልእና ሌሎች ምግቦች					
የቅባት እህሎች ሰሊጥ ተልባ፣ኑግ፣ሱፍ፣ኦቾሎኒ እና ከነዚህ የተሰሩ የተለያዩ ምግቦች					
ወተትና የወተት ተዋጽኦ የሆኑ እርጎ ፣ትኩስ የላም ወተት ፣አይብ ፣የዱቄት ወተት፣አሬራ፣ አጓት እና የመሳሰሉት					
የስጋ (የፍየል፣የበግ፣የከብት፣የዶሮ፣የአሳ፣)እንዲሁም ሌሎች የስጋ አይነቶች ለምሳሌ ጥሬ ስጋ ፣ጥብስ፣ቅቅል፣ክትፎ፣እና ሌሎች ተዋጸኦች በተለያየ አዘገጃጀት					
እንቁላል እና ከእንቁላል የተሰሩ እንቁላል ስልስ እንቁላል ፍርፍር፣ሳንዱች፣የተቀቀለ እንቁላል እና ሌሎች					
በቫይታሚን ኤ የበለጸጉ አረንጓ ተክሎች ጥቅል ጎን፣የሃበሻ ጎመን፣ አረንጓዴ ሰላጣ፣ቃሪያ፣ቆስጣ፣ብሮኮሊ እና ከመሳሰሉት የተሰሩ ምግቦች					
ሌሎች በቫይታሚን ኤ የበለጸጉ የአትክልትና ፍራፍሬ ዘሮች ቲማቲም፣ካሮት፣ቢጫ ድንች፣ፎሶሊያ፣ፓምኪን፣የበሰለ ማንጎ ፣ፓፓያ፣ቢጫ ሙዝ፣አፕሪኮት፣ እና ሌሎች					
ሌሎች ፍራፍሬ ዝዝያዎች ጥሬ ማንጎ፣ጥሬ ፓፓያ፣አቮካ፣ኮኮናት፣ነጭ ሙዝ እና ሌሎች					
ሌሎች የአትክልት ዘሮች ኩከንበር፣አክራ እሸት					

3. የመጠጥውሃመገኛዎችየተመለከቱጥያቄዎች

1. የቤተሰቡዋነኛየመጠጥውሃየሚያገኘውከየትነው?
    1. የቧንቧውሃ
    2. የምንጭውሃ
    3. የወንዝ ፣የግድብወይምየኩሬውሃ
    4. የዝናብውሃ
    5. ከሌላቦታ
2. የመጠጥውሃለማግኘትምንያህልርቀትይጓዛሉ (ሲሄዱናወደቤትሲመለሱ)?

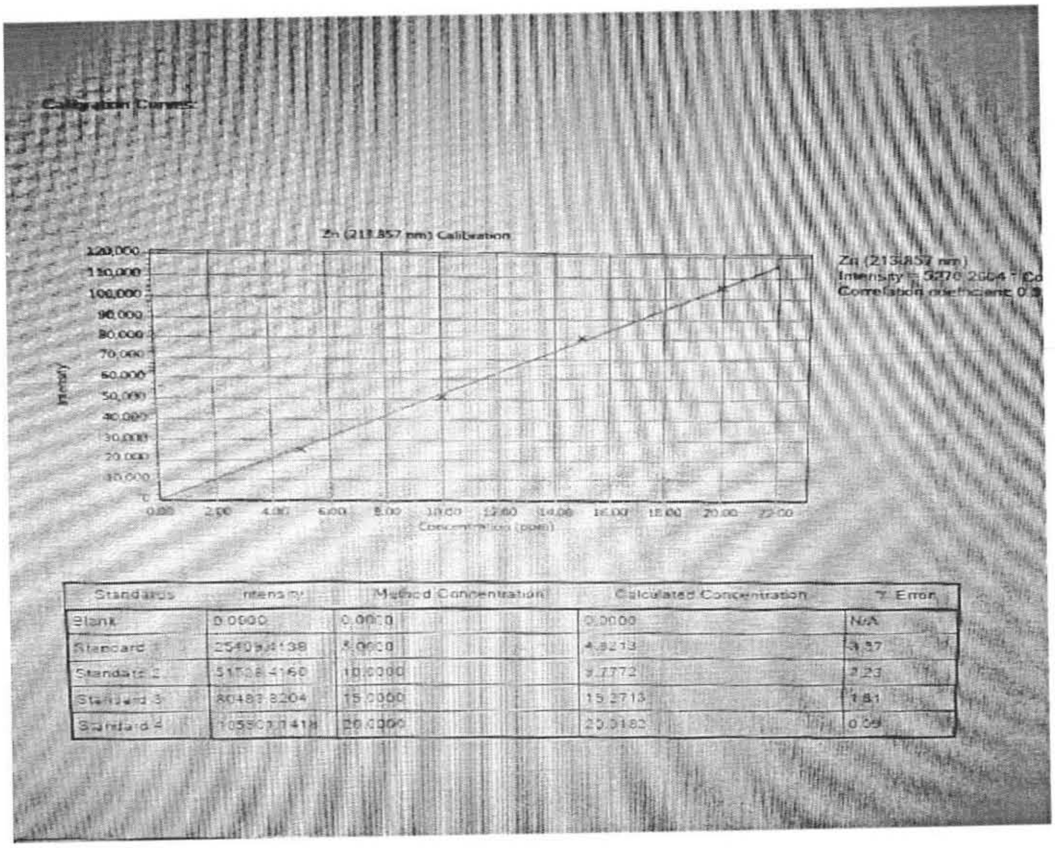
    የሚቆዩበትንሰዓትአይጨምርም

    ____________ደቂቃ
    1. በእርምጃ
    2. በባጃጅ
    3. በጋሪ
3. ለመጠጥየሚቀሙበትንውሃንጹህለማድረግየሚጠቀሙትዘዴአለ?
    1. አይየለም
    2. አዎአለ
    3. አላውቅም
4. የ3ኛው ጥያቄመልስአዎከሆነየሚጠቀሙትዘዴምንድንነው ?
    1. ውሃውንበማፍላትበማቀዝቀዝ
    2. የተለያዩየውሃማጣሪያንጥረነገሮችን (ውሃአጋር፣አኳታብ)በመጨመር
    3. ክሎሪንበመጨመር
    4. በጨርቅ፣ በነጠላበማጥለል
    5. ሴራሚክማጣሪያበመጠቀም
    6. ለተወሰነጊዜየተቀባውንውሃበማስቀመጥበራሱእንዲጠልበማድረግ
    7. በሌላዘዴ

4. ባለፉት 24 ሰዓትዉስጥየተመገቡትንየምግብድግግሞሽንየተመለከቱጥያቄዎች

1. ባለፉት 24 ሰዓታትየስራስርምግቦችእንዲሁምከአገዳእህል (ሰንበሌጥ) ሰብሎችማለትምከገብስ፣ስንዴ፣ማሽላ፣በቆሎ፣አጃ፣ጤፍ፣ዳጉሳእናሌሎችከነዚህሰብሎችየተሰሩምግቦችእንደ እንጀራ፣ዳቦ፣ቂጣ፣ንፍሮ፣ገንፎእናየመሳሰሉምግቦችተመግበዋል?
   1.አዎ ☐ 2. አልበላሁም ☐
2. ባለፉት 24 ሰዓታትከጥራጥሬእህሎች (ምስር፣አተር፣ሽንብራ፣ባቄላ፣አደንጓሬ፣ጓያእንዲሁምከነዚህየእህልዘሮችየተሰሩእንደክክወጥሽሮወጥቆሎን ፍሮወይምሌሎችምግቦችበልተዋል?
   1. አዎ ☐ 2. አልበላሁም ☐
3. ባለፉት 24 ሰዓታትወተትእናየወተትተዋጽኦየሆኑእንደእርጎአይብአሬራአጓትትኩስወተትዱቄትወተትጠጥተዋል (በልተዋል)?
   1. አዎ ☐ 2. አልበላሁም ☐
4. ባለፉት 24 ሰዓታት (የበግ፣የፍየል፣የከብት፣የዶሮ፣አሳ)ስጋእናከነዚህየተሰሩምግቦችእንደጥብስ፣ቅቅል፣ቀይወጥ፣ክትፎ፣ጥሬስጋየ መሳሰሉምግብበልተዋል?
   1. አዎ ☐ 2. አልበላሁም ☐
5. ባለፉት 24 ሰዓታትእንቁላልእንዲሁምከእንቁላልየተሰሩምግቦችእንደእንቁላልስልስ፣እንቁላልፍርፍር፣እንቁላልሳንዱ ች፣እናሌሎችምግቦችበልተዋል?
   1. አዎ ☐ 2. አልበላሁም ☐
6. ባለፉት 24 ሰዓታትበቫይታሚን ኤ የበለጸጉአረንጓዴቀለም,ያላቸውየአትክልትዘሮችእንደየሀበሻጎመን፣ጥቅልጎመን፣ቃሪያ፣ቆስጣ፣አረንጓዴሰላ ጣ፣ብሮኮሊእናሌሎችከነዚህአትክልቶችየተሰሩምግቦችንበልተዋል?
   1. አዎ ☐ 2. አልበላሁም ☐
7. ባለፉት24 ሰዓታትሌሎችበቫይታሚን ኤ የበለጸጉአትክልትናፍራፍሬዎችለ☐ቢጫድንች ፣ካሮት፣የበሰለማንጎ፣የበሰለፓፓያ፣ሚሎን ፣ቢጫሙዝ፣ ሀባብእናሌሎችከነዚህአትክልትናፍራፍሬየተሰራጭማቂወስደዋል (ጠጥተዋል)?
   1. አዎ ☐ 2. አልበላሁም ☐
8. ሌሎችየአትክልትዘሮችለምሳሌቲማቲም ፣ኩከምበር፣ኦክረ፣ሽንኩርትእናሌሎችከነዚህአትክልቶችየተሰሩምግቦችተመግበዋል?
   1. አዎ ☐ 2. አልበላሁም ☐
9. ሌሎችየፍራፍሬዘሮችለምሳሌነጭሙዝ፣ብርቱካን፣ጥሬማንጎ ፣ጥሬፓፓያ፣ አቮካዶ ፣አናናስ፣ፖም፣ትርንጎእናከነዚህየተሰራጭማቂወስደዋል(ጠጥተዋል)?
   1. አዎ ☐ 2. አልበላሁም ☐
10. ባለፉት 24ሰዓታት የቅባትእህሎች (ሰሊጥ፣ኑግ፣ሱፍ፣አቾሎኒ፣ተልባእናከነዚህእህሎችየተሰሩምግቦችተመግበዋል (በልተዋል)?
   አዎ ☐ 2. አልበላሁም ☐

Annex 6: zinc calibration curve



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