Identification of risk factors for anemia on third trimester pregnant women

West Showa Zone, Ambo, Ethiopia

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DECLARATION

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

Signature: _____ Date: _____

The proposal has been approved for submission by:

Name of Supervisor	Signature	Date
Kaleab Baye (PhD) (Advisor)	_____	_____

Abstract

Anemia is a widespread condition leading to adverse pregnancy outcomes. Iron deficiency is often expected to be the cause and thus routine Iron-folic acid (IFA) supplementation is recommended by the World Health Organization (WHO), little or no studies have looked at the etiology of anemia in Ethiopia. The role of other micronutrient deficiencies such as folic acid, vitamin B12, as well as infections in the etiology of anemia remains unknown.

The aim of this study was to investigate the determinants of anemia among third trimester pregnant women (n=208) attending ante-natal care (ANC) in Ambo, Ethiopia.

A cross sectional study was carried out and a semi-structured questionnaire was used to gather socio-demographic, obstetric and nutritional information. Blood samples of 208 mothers were analyzed for hemoglobin (Hb) and blood samples of 147 mothers for serum iron, serum ferritin (SF), transferrin, folate, cobalamin (Vit B12), C-reactive protein (CRP) and α-1-glycoprotein (AGP) levels.

Of the women, 23% had mild (10-10.9g/dl) and 36% had moderate (7-9.9g/dl) anemia. Iron deficiency as assessed by serum iron level of <0.33mg/l was 19%, 26.5% had depleted iron store (SF<15ng/ml), 21.8% and 13.6% had folate and cobalamin deficiencies, respectively. Median iron and folate levels were significantly different among anemic and non anemic pregnant women (P<0.05). The women who were not using iron-folate supplement and were consuming tea or coffee immediately after meal had a 2.9 and 1.6 times higher risk of developing anemia, respectively. Consumption of flesh foods (P=0.02) and dairy products (P=0.04) were significantly associated with improved hemoglobin status.

Consumption of tea, coffee, not taking IFA supplementation and dairy and flesh foods are the main contributors of anemia during pregnancy. Efforts to improve the accessibility and efficiency of the routine IFA supplementation should be exerted. Nutrition education to pregnant women should promote the consumption of dairy and flesh foods during pregnancy while discouraging the consumption of tea and coffee.

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MAY GOD BLESS YOU ALL

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Acronyms and Abbreviations

AGP	1-acid glycoprotein
ANC	Antenatal care
AOR	Adjusted odds ratio
CDC	Center of Disease control and prevention
CI	Confidence interval
COR	Crude oddsratio
CRP	C-reactive protein
CSA	Central Statistical Agency
Dcytb	Duodenal cytochrome b
dl	Deciliter
DMT 1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
EDHS	Ethiopian Demographic Health Survey
ELISA	Enzyme Linked Immunosorbent assay
EPHI	Ethiopian Public health Institution
FAD	Folic acid deficiency
FD	Ferritin deficiency
FDSN	Food science and Nutrition
Fe	Iron
g	Gram
Hb	Hemoglobin
HCP1	Heme carrier protein 1
ID	Iron deficiency
IDA	Iron deficiency anemia
IFA	Iron-Folic acid
l	Litter
MCH	Maternal and child health
MCV	Mean corpuscular volume
mg	Milligram
mL	Milliliter
MTHFR	methyltenetrahydro folate reductase
ng	Nanogram
OR	odds ratio
pg	Picogram
SD	Standard Deviation
SF	Serum ferritin
SPSS	Statistical package Software System/ solution
Vitamin B12	Cobalamin
WHO	World Health Organization

CHAPTER I- INTRODUCTION

1.1 Background

Anemia is defined as the condition in which there is decreased level of hemoglobin (Hb) or red blood cells than the normal value (Eltayeb *et al.*, 2014). According to the 2008 World Health Organization (WHO) report, anemia affected 1.62 billion (24.8%) people globally (WHO, 2008). Approximately 51% of pregnant women were expected to be anaemic in 2001(G.C.). The prevalence in developing countries was 56% and 18% in developed countries (Bick *et al*., 2006). The most recent global estimates suggest that the prevalence of anemia is 41.8% among pregnant women and 30.2% among non-pregnant women (WHO, 2008; Bhutta *et al*., 2012). In Africa, 57.1% of the pregnant women were anaemic. According to Ethiopian Demographic Health Survey (EDHS) report of 2011, the numbers are stated to be lower putting the prevalence of anemia in pregnant women at 22% at the country level and 24.9% in Oromia Regional State encompassing the study area (CSA, 2011).

The risk of anemia is high in women who are premenopausal and/or pregnant. This is mainly due to menstrual blood loss, increased demand to support the fetus, puerperal blood loss, and it is usually exacerbated by nutritional insufficiency that is common in developing countries (Bick *et al*., 2006). Anemia contributes to almost 120,000 maternal deaths globally and indirectly to almost a fifth (18%) of the burden of maternal mortality. In addition to maternal deaths, there are several adverse health outcomes associated with anemia including preterm delivery, intrauterine fetal death, stillbirths, neonatal mortality, low birth weight (LBW) and poor cognitive development in the offspring (Bhutta *et al.*, 2012; Melku *et al*., 2014).

In developing countries, maternal anemia during pregnancy is a product of many factors, such as maternal malaria, intestinal parasitic infection, recurrent infection, reduced dietary intakes, parity and micronutrient deficiencies just to name but few (Bhutta *et al.*, 2012; Ugwuja, *et al.*, 2011; Haidar, 2010). Among the micronutrient deficiencies the most common encountered are iron deficiency anemia (IDA) and folate deficiency

megaloblastic anemia. Of all anemia diagnosed during pregnancy, 75% are due to iron deficiency. Iron deficiency (ID) alone affects nearly 20% of the world's population (Bick *et al*., 2006). The odds for developing anemia were 60% more likely in the iron-deficient and 40% more likely in the folic acid deficient. One in every three women had anemia and deficiency of folic acid while one in every two had iron deficiency, suggesting that deficiencies of both folic acid and iron constitute the major micronutrient deficiencies in Ethiopian women (Haidar, 2010). Apart from iron and folate deficiency cobalamin (Vit B12) deficiency has been shown to account for some cases of anemia (Morris *et al*., 2007; Khan *et al*., 2010).

Studies show that iron intake in Ethiopia is adequate (Gibson *et al*., 2008), however anemia is still a health concern affecting 22% of pregnant women (WHO, 2008) leading to devastating outcomes of pregnancy and delivery necessitating studies to be conducted on other micronutrients which could play an important role on the occurrence of anemia. In the present study the socio-demographic and obstetric characters and plasma levels of iron, folate and cobalamin, was evaluated in anaemic (case) and non-anaemic (control) pregnant women to ascertain the impact these factors have on maternal anemia and the possible interactions among them.

1.2 Statement of the problem

Anemia is a major public health problem all around the world and even more in developing countries like Ethiopia with a prevalence of 22% (CSA, 2011). Anemia could be the consequence of many factors such as: infections, micronutrient deficiency, blood loss or genetic disorders (Bhuta *et al*., 2012; Ugwuja, *et al.,* 2011; Haidar, 2010). Of all the micronutrients that are presumed to be predisposing factors ID has been extensively assessed but others like folate and cobalamin, should also be assessed as they have been shown to have great impact on development of anemia on researches done on other countries (Gibson *et al*., 2008; Khan *et al.,* 2010; Knovich *et al*., 2008). The purpose of this study was to investigate and compare the Iron, Folate and Cobalamin status among anaemic and non anaemic third trimester pregnant women attending Antenatal care (ANC) in West Showa zone of Oromia regional sate to generate evidence that can potentially improve maternal health.

1.3 Objective of the study

1.3.1 General objective

- ✓ Assess the etiology of anemia among pregnant women in their 3rd trimester of pregnancy

1.3.2 Specific objective

- ✓ Investigate the association between socio-economic, dietary and micronutrient status and anemia
- ✓ Assess the possible effect of dietary characteristics on micronutrient status

CHAPTER II- LITERATURE REVIEW

2.1 Anemia and pregnancy

Pregnancy is a period of increased requirements for micronutrients to fulfill demands for physiological changes of mother and the fetus. During this period, micronutrients such as iron, folic acid and cobalamin, are the major marker that affects both the fetus and the pregnant women. Utilization of one nutrient is often dependent on the adequate supply of another nutrient and deficiency of any one of them may affect biochemical functions of the other (Eltayeb, 2014; Bakeit *et al*., 2011).

As defined by the WHO, anemia is present when the Hb level decreases to less than 12g/dl for premenopausal and prepubertal women and Hb of less than 13g/dl for men and postmenopausal women. Anemia in pregnancy is generally described as Hb less than 11g/d due to the increase in maternal plasma volume by approximately 42% above the pre-pregnant state which is disproportionately greater than the corresponding increase in red cell mass (WHO, 2008; Bick *et al*., 2006).

2.2 The effect of selected micronutrients on anemia

2.2.1Iron

Iron (Fe) plays a vital role in oxygen transport and storage, oxidative metabolism, cellular proliferation and many other physiological processes. Its most important property is the reversible one electron oxidation-reduction reaction between the two common oxidation states, Fe2+ and Fe3+, allowing it to coordinate electron donors and to participate in redox processes. Human beings normally have 40–50mg Fe/kg body weight. Approximately 75% is present in metabolically active compounds. The remaining 25% constitutes a dynamic store that is turned over constantly. It ensures an adequate supply for normal physiological functions despite short term variations in absorption or loss from the body. The store also supplies the immediate needs when requirements are increased (e.g., during rapid growth or pregnancy). Iron reserves that have been utilized are then gradually replaced by increased absorption (Lynch, 2007).

Ferritin is the major iron storage protein. It is located predominantly in the cells that function as the storage sites, the macrophages of the spleen, liver, bone marrow and skeletal muscle. However, all nucleated cells synthesize ferritin to manage their intracellular iron economy (Halliday *et al*., 1994). Each ferritin molecule can reversibly store as many as 4,500 iron atoms within the protein shell. Channels that connect the interior with the surface provide routes for iron to move in and out in concert with cellular requirements. Catabolism of ferritin may result in the utilization of the iron core or conversion to hemosiderin which is an amorphous form of iron that is water insoluble and less rapidly available (Lynch, 2007; Wixom *et al*., 1980).

Transferrin is iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids by binding to iron tightly, but reversibly. Although iron bound to transferrin is less than 0.1% (4 mg) of total body iron, it forms the most vital iron pool with the highest rate of turnover (Yang *et al*., 1984). When a transferrin protein loaded with iron encounters a transferrin receptor on the surface of a cell, it binds to it and, as a consequence, is transported into the cell. The liver is the main site of transferrin synthesis but other tissues and organs, including the brain, also produce transferrin. The main role of transferrin is to maintain iron homeostasis in the cells by controlling iron concentrations. An increased plasma transferrin level, >3.1g/l, (Punnonen *et al*., 1997) is often seen in patients suffering from IDA, during pregnancy, and with the use of oral contraceptives, reflecting an increase in transferrin protein expression (Miller, 2013).

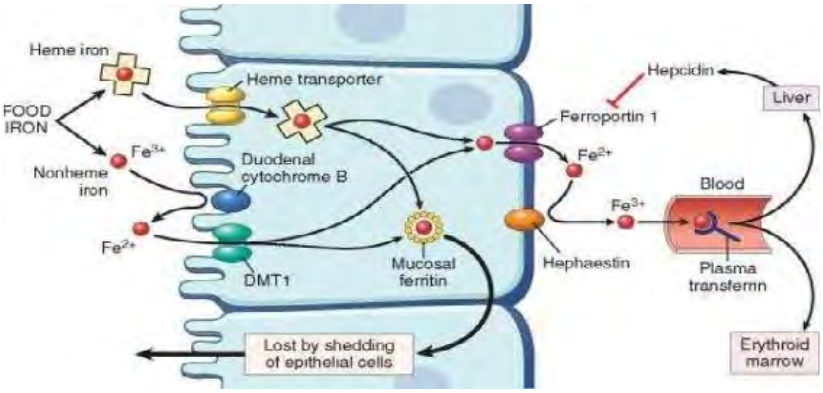
C-reactive protein (CRP) and Alpha-1 acid glycoprotein (AGP) are the classical acute phase reactant, the circulating concentration of which rises rapidly and extensively in a cytokine-mediated response to tissue injury, infection and inflammation. Elevated level of CRP (>5mg/l) and AGP (>1g/l) indicates inflammation. These values are used to adjust ferritin value increment due to inflammation (Thompson, 1999; Fournier *et al*., 2000).

The cells in most body organs are turning over constantly, necessitating a steady supply of nutrients, including iron. The iron requirements of the bone marrow for Hb synthesis outweigh those of all other tissues from a quantitative point of view. Almost all

functional requirements are supplied from the circulating transferrin bound pool which contains only about 3 mg iron in adults but ten times as much iron, approximately 35 mg, moves through the compartment each day, roughly 80% destined for red blood cell production (Finch and Huebers, 1982).A small proportion of the iron passing through the plasma transferrin pool, about 1 mg, is absorbed iron. The largest fraction is iron recovered from the turnover of erythrocytes and defective erythrocyte precursors. At the end of their 120 day life spans, red blood cells are catabolized by specialized macrophages in the spleen, liver and bone marrow. Depending on the body's immediate requirements, the recovered iron is either released to transferrin within a few hours or temporarily placed in the cellular store. Finally, a variable but much smaller quantity of iron is derived from liver hepatocytes, which constitute another important storage site (Fillet *et al.*, 1989). The body's ability to provide additional iron by increasing absorption is very different. It is limited to about 2–4 mg per day unless iron supplements are provided (Lynch, 2007).

2.2.1.1 Absorption

Iron is a constituent of most foods. However, its availability for absorption is quite variable among heme and non-heme iron and poor bioavailability is the major reason for the high prevalence of nutritional IDA in developing countries. Absorption occurs primarily in the proximal small intestine (duodenum) through mature enterocytes located on the tips of the intestinal villi. Two transporters appear to mediate the entry of most if not all dietary iron into the mucosal cells; one transfers intact heme molecules derived from Hb or myoglobin in meat (heme carrier protein 1 (HCP1)) (Shayeghi *et al*., 2005) and the other (divalent metal transporter 1 (DMT 1)) all other iron that is rendered soluble in gastric juice and remains in solution in the upper small intestine (non-heme iron). Absorption of non-heme iron requires reduction to the ferrous state by dietary components such as ascorbic acid and/or mucosal ferric reductases, duodenal cytochrome b (Dcytb) being considered the most important.


Heme iron
Heme transporter
FOOD IRON
Nonheme iron
Fe^{3+}
Duodenal cytochrome B
Fe^{2+}
DMT1
Mucosal ferritin
Lost by shedding of epithelial cells
Hepcidin
Liver
Ferroportin 1
Fe^{2+}
Fe^{3+}
Hephaestin
Blood
Plasma transferrin
Erythroid marrow

2.2.1.3 Excretion

Iron is lost from the body through the exfoliation of skin cells, in sweat and urine and through the gastrointestinal tract. These daily basal losses account for about 1.0 mg and 0.9 mg in men and women respectively. Menstrual losses (averaged over 30 days) add a mean of 0.60–0.70 mg in women of childbearing age (Lynch, 2007).

2.2.1.4 Iron requirements and food source

Dietary iron requirements are highest in the second and third trimesters of pregnancy and in the rapidly growing infant between 6 and 18 months of age. The next high risk period for nutritional ID is the adolescent growth spurt and the onset of menstruation in girls. Iron requirements are least in adult men and post-menopausal women (Lynch, 2007). Large amount of iron (840mg) is used during pregnancy and delivery and up to 2 years of normal dietary intake is required to replace the loss and over 500mg of storage is required to avoid ID during pregnancy. Demand for iron increases from 0.8mg/day early in pregnancy to 7.5mg/day in late pregnancy to supply fetal iron needs and to withstand the physiologic challenge of puerperal blood loss. Neonatal iron stores are dependent upon those of the mother. ID during the first two trimesters of pregnancy doubles the risk of pre-term delivery triples the risk of neonatal low birth weight and results in the delivery of iron deficient neonates. The World Health Organization dietary allowance for iron, based upon current knowledge of normal iron homeostasis, is 5 to 10mg/day. Iron can be acquired from clams, liver, sunflower seeds, nuts, beef, lamb, beans, whole grains, dark leafy greens (spinach), dark chocolate, and tofu (Bick *et al*., 2006; Muslimatun *et al*., 2001).

2.2.1.5 Iron deficiency

Iron is the most extensively investigated micronutrient that is considered lacking in the diets of pregnant women. This is because anemia, attributable to ID is a major problem in developing countries and even in developed countries (Eltayeb, 2014; Bakeit *et al*., 2011). Diets with an inadequate content of bioavailable iron are the primary cause, but

disorders that increase iron loss as a result of pathological bleeding, particularly hookworm, have a very important role. Malabsorption due to disorders that affect the upper small intestine can also cause ID. The most common appear to be celiac disease and Helicobacter pylori infection. Surgical procedures that alter the anatomy of the stomach and duodenum may also decrease iron absorption (Lynch, 2007). Since ID usually responds to iron supplementation or fortification, the assessment of iron status is crucial in the evaluation of nutritional anemia (Biesalski and Erhardt, 2007).

Specific symptoms and signs may suggest IDA. Pica is reported in as many as 50% of patients as a symptom of severe ID. Pallor is the physical finding most often observed in patients with IDA (Bick *et al*., 2006). Usually IDA occurs in three sequentially developing stages: depleted iron stores, iron deficient erythropoiesis and IDA as shown in **Table 1.** These stages can be analyzed biochemically and there is now an agreement that the measurement of Hb, ferritin and transferrin, complemented with indicators of acute and chronic infections, is the best procedure for evaluating iron status (Biesalski and Erhardt, 2007).

Comment [O1]: Is this a proven association? Are these the only s/s of anaemia??

Table1: Influence of the iron status on various indicators (Biesalski and Erhardt, 2007)

	Hb	Ferritin (µg/L)
Iron overload	Above cut off	> 300
Normal	Above cut off	100 ± 60
Depleted iron stores	Above cut off	20
Iron deficient erythropoiesis	Above cut off	10
Iron deficiency anemia	Below cut off	< 10

For the diagnosis of anemia, it is essential to measure Hb in blood. It is one of the most common and least expensive measurements. The cut off values of Hb to define anemia, as stated by WHO, are shown on the **Table 2**.

Table 2: Cut offs for Hb to define anemia (WHO, 2008)

	Cut off
Children aged 0.5-5 years	<110 g/L
Children aged 5-11 years	<115 g/L
Children aged 12-13 years	<120 g/L
Men	<130 g/L
Non-pregnant women	<120 g/L
Pregnant women	<110 g/L

Currently the most important indicator for the iron status is the measurement of ferritin. The plasma level correlates well with the iron stores, and in the first stage of ID the concentration of ferritin already decreases, which makes it the most sensitive parameter. Low ferritin always indicates iron storage depletion. ID is diagnosed when the serum ferritin is ≤12mg/l and excluded when >12mg/l (Bick *et al.*, 2006; Gibson *et al.*, 2010). Since ferritin is increased by a number of factors, especially infection and inflammation, a high value is not inevitably a sign of a good iron status. To solve this problem it is helpful to also measure parameters for acute and chronic infection, to identify subjects in which the ferritin concentration might be increased by infection. Currently the most used parameter for the assessment of acute infection or inflammation is the determination of CRP. Elevation of this >5 mg/L in serum suggests that an elevated serum ferritin may be due to acute inflammation (Bick *et al*., 2006; Gibson *et al.*, 2010). Chronic infections can be assessed by measuring serum AGP. AGP has a normal plasma concentration between 0.6-1.2 mg/mL (Colombo *et al*., 2006), which is less influenced by infection and can also used to assess IDA in the presence of inflammation. The concentration is increased in the second stage of ID. Usually an Enzyme Linked Immunosorbent assay (ELISA) or turbidimetric technique is used to measure transferrin (Biesalski and Erhardt, 2007).

2.2.2 Folate and Cobalamin

Folate and cobalamin are members of the B vitamins and functions in maintaining deoxy ribonuclic acid (DNA) synthesis and cell division.

2.2.2.1 Absorption and Transportation

Food delivers folate mostly in the "bound" form (attached to amino acids) known as polyglutamate. The small intestine prefers to absorb the free folate there for enzymes on the intestinal cell surface hydrolyze the polyglutamate to monoglutamate and several glutamates. Then the monoglutamate is attached to a methyl group. For the folate coenzyme to function the methyl group must be removed and this requires the help of cobalamin. Without the help folate becomes trapped in its methyl form inside cells. When folate gives up its methyl group, the cobalamin coenzyme becomes activated. In the stomach, HCL and digestive enzyme pepsin releases cobalamin from the protein it is attached to in food. The stomach also secrets a molecule called intrinsic factor (IF). As cobalamin passes to the small intestine, it binds to IF and is recognized by receptors. Then the IF is degraded and cobalamin is gradually absorbed into the blood stream (Ellie & Sharon, 2011; Scott, 1999).

Both folate and cobalamin follow the enterohepatic circulation route where they are continuously secreted in to bile and delivered to the intestine where they are reabsorbed (Ellie & Sharon, 2011).

2.2.2.2 Daily requirement and food source

There is a large increase in requirement of these vitamins during pregnancy and lactation associated with the rapid extra demand for DNA synthesis during which a significant extra amount is catabolized (Higgins *et al.*, 2000). The current recommended daily intake for pregnant women is 400µg folate and 2.6µg cobalamin (Ellie & Sharon, 2011; Stover, 2010).

Folate can't be synthesized in the human body, but is fortunately available from a wide variety of food sources including organ meats, leafy green vegetables, citrus fruit, bread, and dairy products. Cooking and ultraviolet radiation destroy the folate in many food sources (Bick, 2006). Cobalamin is unique among the vitamins in being found almost exclusively in foods derived from animal source (Watanabe, 2007).

2.2.2.3 Deficiency

Deficiency of folate and cobalamin becomes most apparent through their functions in maintaining purine and pyrimidine biosynthesis. A reduction in ability to synthesize deoxy ribonuclic acid (DNA) and maintain cell division is most easily seen in the synthesis of red cells, expressed as a very characteristic macrocytic megaloblastic anemia. Deficiency of these vitamins would reduce the ability of any cell to divide appropriately but would be most obvious clinically in the rapidly dividing cells of the bone marrow, which causes an anemia. As red cells divide in the bone marrow compartment, the resultant two daughter cells after each division are slightly smaller than the parent cell. The reduction in the number of such divisions results in the eventual erythrocytes being larger than usual, or macrocytic, with a raised mean corpuscular volume (MCV) (Scott, 2007; Weir and Scott, 1995).

The Causes of deficiency of these vitamins may be classified into three broad categories: decreased intake, impaired absorption and increased requirements. In general, folate deficiency is most often the result of decreased intake and cobalamin deficiency is due to malabsorption due to gastro intestinal abnormality or after gastric surgery. This is due to the absence of acid preventing the liberation of cobalamin from the bound form that it is present in foods. But it also occurs in individuals who are strict vegetarians or vegans who do not consume any animal products. This is due to the enzymes necessary to assemble this very large vitamin are only present in bacteria and some algae. Its synthesis is completely absent in plant and vegetables of all kinds. cobalamin enters the human food chain exclusively through animal products, either as meat or milk, milk products or eggs, therefore, vegetarians or more particularly vegan communities are at higher risk of being cobalamin deficient (Scott, 2007; Green and Miller, 1990).

Appropriate treatment of folate deficiency, either with food folate but more usually with the synthetic form of the vitamin folic acid, produces a complete remission of the anemia. Likewise, treatment of cobalamin deficiency with cobalamin produces a complete remission of the anemia. If the cobalamin deficiency is nutritional, as in a vegan's, additional cobalamin, either by way of fortification or more usually by supplements, can be effective. If the malabsorption is due to absence of the intrinsic factor needed for absorption, then cobalamin treatment must be parenteral (Scott, 2007).

Comment [B2]: You have not stated what foods are rich and not…. So talking about Vegans' diet at this stage is too early…

Accepted cutoff values indicating deficiency is serum/plasma cobalamin level below 200pg/mL (148pmol/L) and serum folate levels below 7nmol/L (3ng/mL) (Ellie and Sharon, 2011). The approach to the treatment of folate and cobalamin deficiency depends on the clinical situation in which the diagnosis is suspected. When a megaloblastic anemia is the initial presenting problem, the anemia itself may be the most important symptom and transfusion of packed red blood cells may be indicated. To treat the root cause, folate deficiency is effectively treated with folic acid supplements between 1–5mg daily and cobalamin deficiency with 0.4 to 1mg of cobalamin daily (Bick *et al*., 2006)

CHAPTER III- MATERIAL AND METHODS

3.1 Study design

A health facility based cross-sectional study was carried out among pregnant women (n=208) in Ambo, Oromia region.

3.2 Study area

The present research work was carried out at Ambo, Western Shoa zone of Oromia regional state. Ambo has latitude and longitude of $8^0$59'N $37^0$51'E and an elevation of 2101 meters above sea level and a total of 48,171 residents. It has an annual rain fall ranging from 800–1000 mm, temperature of 23°C and 47% humidity.

3.3 Source and study Population

The source population of the study were third trimester pregnant women who attended ante-natal care (ANC) at Ambo hospital and two selected health centres. The women that resided in the study area, and were willing to give blood were included. The exclusion criteria was having pregnancy-related complications including gestational diabetes, hypertension. If women had a blood transfusion within the last three months, they were excluded.

3.4 Sample size and sampling technique

Convenience sampling method was used to select 208 pregnant women (104=anemic and 104=non anemic) from 3 health institutions selected from 3 kebeles using the random sampling technique. A proportional allocation was employed to obtain the sample size from each health institutions. Gestational age was assessed from the reported last menstrual period and examination of fundal height and was expressed in weeks by experienced midwives at maternal and child health (MCH) centre of each health institution.

3.5 Ethical consideration

The research proposal was approved by the Ethical Clearance Committee of the College of Natural Sciences and was further approved by the Oromia Health Research Ethical Review Committee before data collection was started. A supportive letter was written by the Oromia regional health Bureau to West Showa Zone health office and Hospital. All participants were consulted and verbal informed consent was obtained.

3.6 Sample Collection and Methods

3.6.1 Socio-demographic, dietary and obstetric characteristics

A semi-structured questionnaire was used to gather socio-demographic information like age, education, marital status, place of delivery, blood loss, interval between babies, contraceptive use, etc. Using 24 hours recall method; respondents were asked whether they had taken any food from predefined food items related to iron, folate and cobalamin such as consumption of animal source foods and dairy products in the previous day of the survey and beverages like tea and coffee after meal were recorded. Obstetric history of the participants was also assessed. The questionnaires were translated to Afan oromo and back-translated to English for analysis.

3.6.2 Blood sample collection

Venous blood sample (~4 ml) was drawn into vacutainer tube without anticoagulants by medical laboratory technicians or well-trained phlebotomists. A few drops were used for Hb analysis using a hemoglobinometer (Hemocue 201) and the remaining was transferred to a gel tube that was allowed to clot and be centrifuged at 3000 rpm, for 15 min and the serum was recovered for further biochemical analysis. Then the extracted serum was transferred into labeled screw-top vials and frozen at -20℃ until analysis. The stored samples were transported to Ethiopian Public Health Institute (EPHI) for biochemical analysis of serum iron, transferrin, ferritin, CRP, AGP, cobalamin and folate.

3.6.3 Blood sample analysis

3.6.3.1 Hemoglobin test

Hemoglobin test was performed from whole blood using a portable hemoglobinometer (Hemocue) device and the reading was adjusted for altitude according to the WHO Hb adjustment formula described below (Nestel, 2011).

Hb adjustment = -0.032 x (Altitude x 0.0033) + 0.022 x (Altitude x 0.0033)2

Hb adjustment = -0.032 x (2101 x 0.0033) + 0.022 x (2101 x 0.0033)2= 0.82

The adjustment value (0.82) was subtracted from each Hb value. WHO recommended Hb concentration cut-offs value for pregnant women was used, where anemia was defined as Hb value <11g/dl and severe, moderate, and mild anaemia were defined as Hb level below 7g/dl, 7-9.9g/dl and 10-10.9g/dl, respectively.

Laboratory analysis

After thawing the stored sample to room temperature, a sample of 300µl was transferred to the instrument's (Cobas 6000 analyzer series, Germany) sample holders and serum iron, ferritin, transferrin, CRP, AGP, folate and cobalamin concentrations were analyzed. The cobas 6000 analyzer series (fig 2) uses two methods for the analysis of the micronutrients investigated in this research. Clinical chemistry method was used to analyze iron, transferrin, AGP and CRP, and electro chemiluminesence assay method was used for ferritin, folate and cobalamin analysis.

Fig 2: Cobas 6000 analyzer series

3.6.3.2 Determination of serum iron concentrations

Colorimetric assay test principle for iron detection was based on the liberation of Fe^{3+} ions from the transferrin complex using acids or detergents (Citric acid), reduction to Fe^{2+} form by sodium ascorbate and reaction of the Fe^{2+} ions to give a colored complex. A simpler illustration of the process is shown bellow. The final reading is done by using a wavelength (sub/main) of 700/570 nm.

transferrin Fe complex $\xrightarrow{pH < 2.0}$ apotransferrin + Fe^{3+}

$Fe^{3+} \xrightarrow{ascorbate} Fe^{2+}$

Fe^{2+} + FerroZine $\longrightarrow$ colored complex

The measuring range of the instrument is 0.05 10.0mg/L. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2.1. The results are automatically multiplied by this factor. Values below 0.33mg/l are indicative of ID (Shaikh, 2014).

3.6.3.3 Determination of serum ferritin concentrations

At the 1st incubation, 10µL of sample, a biotinylated monoclonal ferritin specific antibody, and a monoclonal ferritin specific antibody labeled with a ruthenium complex form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin on 2nd incubation. Then the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve. According to Roche diagnostic laboratory the measuring range is 0.500 2000µg/L (ng/mL). Expected values are 30 400µg/L (ng/mL) and 13 150µg/L (ng/mL) for men and women respectively. WHO cut off value of <15µg/L for normal inflammatory marker level and in the presence of inflammation, <30µg/L was used.

3.6.3.4 Determination of serum transferrin concentrations

Anti-transferrin antibodies react with the antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. According to Roch diagnostic laboratory measuring range for transferrin is 0.1 5.0g/L. Values of transferrin exceeding 3.1g/l were considered indicative of ID (Punnonen *et al*., 1997).

3.6.3.5 Determination of serum CRP concentrations

Human CRP agglutinates with latex particles coated with monoclonal anti CRP antibodies. The precipitate is determined turbidimetrically at 552nm. According to Roche diagnostic laboratory, 0.1 20mg/L is the measuring range. Values exceeding 5 mg/L are indicative of inflammation (WHO, 2008).

3.6.3.6 Determination of serum AGP concentration

AGP antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Any value between 0.25 4.0g/l can be measured with the expected normal value being 0.5 1.2g/l values exceeding 1g/l are indicative of inflammation (Bengana *et al.*, 2015).

3.6.3.7 Determination of serum folate concentrations

The bound folate is released from endogenous folate binding proteins to form a folate complex which is then bound to the solid phase. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. According to the WHO serum folate concentration of >20ng/ml is considered as elevated, 6-20ng/ml as normal, 3-5.9ng/ml as moderate and concentration of <3ng/ml as severe folate deficiency (Gibson *et al.*, 2008; WHO, 2008).

3.6.3.8 Determination of Cobalamin concentrations

Cobalamin in the sample competes with the added cobalamin labeled with biotin for the binding sites on the ruthenium labeled intrinsic factor complex. This is achieved in the same manner as folate. The measuring range and normal values are 50.0 2000 pg/ml and 197-866 pg/ml, respectively. Cobalamin deficiency is diagnosed when serum level is <200pg/ml (Higgins *et al*., 2000).

3.7 Statistical analysis

Data entry, screening and analysis were carried out by using SPSS version 20. The normality of the distribution of key continuous variables (Hb, folate, ferritin, transferrin, cobalamin, CRP and AGP) was assessed using various options including statistical tests (Shapiro-Wilk tests) and visual evaluation of histogram and probability plots. When the distribution was not normal, log-transformations were applied.

Data description was made using mean, median, Standard Deviation (SD), frequency, percentage and graphs. Comparisons between anemic and non-anemic pregnant women were made using the student's t-test for parametric data and the Mann-Whitney test for non-parametric data. Correlation coefficients were determined by linear regression analysis. Binary logistic regression was carried out to determine the odds ratio (OR) at 95% CI. In all statistical comparisons significance level was denoted by p value of less than 0.05at α=0.05.

CHAPTER IV- RESULT AND DISCUSSION

4.1 Socio-demographic character of pregnant women

As can be seen on **Table 4.1**, the mean age of the respondents was 24.5±5 years and most 81(38.9%) were between 20-24 years of age. Nearly all, 199(95.7%), of the respondents were married. Concerning educational status, 76(36.5%) were illiterate and 104(50%) had higher education. More than half, 120(57.7%), of the participants had low level of income.

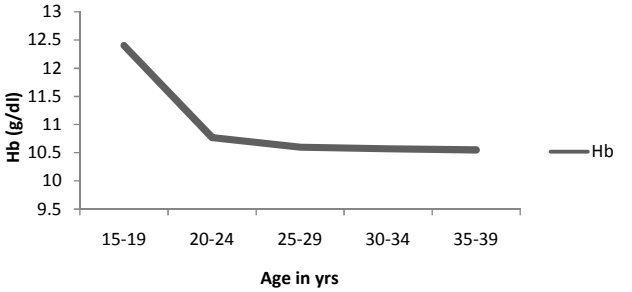
As can be seen on **Table 4.1**, age of participants did not have significant correlation with development of anemia. But Hb level showed a linear decrease with increase in age and decrease in educational level **(Fig 1&2)**. A previous study done by Gebremedhin & Enquselassie, (2011) showed that compared to the youngest group, the risk of anemia is significantly higher in the age group 35-39 years. This effect was not seen in the present study as a result of homogeneity of age of participants where 94.7% of the participants were in the age group <35yrs.

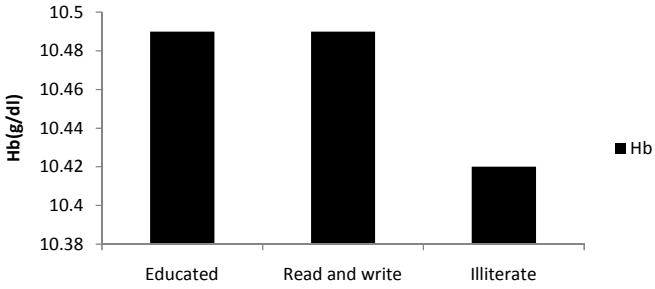
Table 3: Socio-demographic characters of the participants

Characteristics	Frequency N (%)			COR	AOR
	Anemic	Non anemic	Total	95% CI	95% CI
Age	24.8±5.2	24±4.7	24.5±5		
15-19	21	12	33(15.9)	1	1
20-24	56	25	81(38.9)	0.78(0.3-1.8)	0.8(0.3-1.9)
25-29	37	17	54(26)	0.8(0.3-2)	0.84(0.32-2.2)
30-34	20	9	29(13.9)	0.79(0.3-2.3)	0.88(0.3-2.6)
35-39	10	1	11(5.3)	0.18(0.02-1.5)	0.18(0.02-1.6)
>39	-	-	-		
Marital status					
Married	138	61	199(95.7)	1	1
Single	5	1	6(2.9)	0.45(0.05-3.96)	0.45(0.05-4.1)
Other	1	2	3(1.4)	4.5(0.4-50.9)	4.76(0.39-57.3)
Educational Status					
Educated	68	36	104(50)	1	1
Read and write	19	9	28(13.5)	0.09(0.37-2.18)	0.81(0.31-2.1)
Illiterate	57	19	76(36.5)	0.63(0.36-1.2)	0.55(0.28-1.1)
Occupation					
Farmer	48	16	64(30.8)	1	1
House wife	77	38	115(55.3)	1.42(0.7-2.8)	1.3(0.62-2.7)
Merchant	21	10	31(14.9)	1.37(0.5-3.5)	1.37(0.48-3.7)
Level of income					
High	4	2	6(2.9)	1	1
Middle	58	24	82(39.4)	0.83(0.14-4.8)	0.93(0.15-5.7)
Low	82	38	120(57.7)	0.93(0.16-5.3)	1.07(0.18-6.5)
Religion					
Christian	144	63	207(0.99)	-	-
Muslim	-	1	2(1.4)	-	-

All 208 participants are included

Results are expressed as mean ± SD and N(%): number of participants and percentage; COR: crude odds ratio; AOR: adjusted odds ratio; * Significant correlation at 95%CI


13
12.5
12
11.5
11
10.5
10
9.5
Hb (g/dl)
15-19
20-24
25-29
30-34
35-39
Age in yrs
Hb


10.5
10.48
10.46
10.44
10.42
10.4
10.38
Hb(g/dl)
Educated
Read and write
Illiterate
Hb

4.2 Obstetric characteristics of participants

As illustrated on **Table 4.2**, more than half, 116(55.8) of the mothers were multiparas out of which 66(31.7%) delivered their previous babies at home. Most of the mothers, 80(38.5%), had no history of significant blood loss during previous deliveries. Malaria infection was prevalent on 16.8% of the pregnant mothers. The vast majority (53.8%) of participants delivered their babies in more than 2 years interval. In terms of contraceptive use, 60.6% had been using some form of contraception.

In terms of correlation no statistically significant association was noted between these factors and categories of anemia. Contradictory studies have been published on the effect of contraceptive use with regards to development of anemia. On a previous study the women who were not using contraceptive were 1.4 times more likely to develop anemia than contraceptive users (Gebremedhin & Enquselassie, 2011). But other studies showed that folate (Shojania, 1982) and cobalamin (McArthur *et al.*, 2013) levels fell progressively with oral contraceptive usage. However, Castren & Rossi, (1970) and Paine *et al.*, (1975) found no change in folate levels with time on steroid contraceptives. Mountfield, (1985) discussed on his study that the fall in folate and cobalamin level dose not return to normal until about 3 months after usage has stopped which shows that the effect of the contraceptive would not be seen at their trimester of pregnancy.

Although no significant association was seen between pregnancy interval and anemia on the present study due to almost all participants having more than 2yrs inter pregnancy interval, other studies have shown pregnancy interval to have an effect on Hb level (Bakeit *et al.*, 2011; Balarajan *et al.*, 2012).

On the study done at Kampala/Uganda malaria infection was shown to increase the odds of developing anemia by 6.85 times (Baingana *et al.*, 2015). Unlike the present study where malaria infection was assessed using interview method malaria infection was assessed by blood test (on the time of the study) which might be the cause for the discrepancy of result with the current study.

Table 4: Obstetric characteristics of participants

Characteristics	Frequency N (%)			COR	AOR
	Anemic	Non anemic	Total	(95% CI)	(95% CI)
Parity					
Nullyparas	61	31	92(44)	1	1
Multiparas	83	33	116(55.8)	0.78(0.43-0.4)	9.03(0.6-117)
Previous babies delivery place					
Health institution	33	17	50(24)	1	1
Home	50	16	66(31.7)	0.62(0.28-1.4)	0.4(0.16-1.09)
Blood Loss					
No	69	21	80(38.5)	1	1
Yes	15	11	26(13.5)	1.77(0.76-4.1)	4.32(1.5-12.8)
Malaria infection					
No	120	53	173(83.2)	1	1
Yes	24	11	35(16.8)	1.04(0.47-2.3)	1.37(0.56-3.2)
Interval between babies					
>2 years	82	30	112(53.8)	1	1
<2 years	2	2	4 (1.9)	2.87(0.4-21.3)	2.81(0.3-23.6)
Use of Contraceptive					
Yes	93	33	126(60.6)	1	1
No	51	31	82 (39.4)	1.7(0.9-3.1)	1.78(0.9-3.5)

All 208 participants were included

Results are expressed as N(%): number of participants and percentage; COR: crude odds ratio; AOR: adjusted odds ratio

* Significant correlation at $p<0.05$

4.3 Dietary habits of participants across categories of anemia

A significant negative correlation was seen between anemia and IFA supplement use, where the pregnant women who were not using IFA supplement were more likely to develop anemia than those who were using supplements (AOR=2.9, 95% CI 1.4-5.8). This result is in agreement with the result reported by Bakeit *et al.*, (2011), which states that supplementation plays an important role on maternal dietary adequacy affecting both pregnancy and lactation. In the current study odds for developing anaemia was 2.9 times more likely among women not taking iron–folate supplement than their counterparts as shown on **Table 4.3**.

Statistical significant positive correlation was seen between consumption of tea or coffee after meal on daily bases and risk of development of anemia, where those consuming tea or coffee after meal on daily bases has a 1.6 time higher risk developing anemia (AOR=1.6, 95% CI 1.15-54.7).. This is because tea inhibits the absorption of non-heme iron to a significant level (Disler *et al.*, 1975).

Table 5: Dietary habits of participants

Characteristics	Frequency N (%)			COR (95% CI)	AOR (95% CI)
	Anemic	Non anemic	Total		
Supplementation					
IFA	79	23	102(49)	1	1
No supplement	65	41	106(51)	3.16(1.7-5.8)	**2.9(1.4-5.8)***
Flesh foods					
Yes	71	25	96(46.2)	1	1
No	73	39	112(53.8)	0.66(0.36-1.2)	0.8(0.38-1.67)
Dairy Products					
Yes	54	16	70(33.7)	1	1
No	91	47	138(66.3)	0.6(0.3-1.2)	0.8(0.38-1.67)
Ca rich food					
Yes	85	41	126(60.6)	1	1
No	59	23	82(39.4)	1.2(0.67-2.28)	1.2(0.6-2.6)
Tea or coffee					
No	11	4	15(7.2)	1	1
Yes	133	60	193(92.8)	1.8(1.24-4.6)	**1.6(1.15-4.7)***

All 208 participants were included

Results are expressed as N(%); N, number of women in the category; COR: crude odds ratio; AOR: adjusted odds ratio; Ca: calcium; IFA: iron folic acid

* Significant correlation at $p<0.05$

4.4 Serum micronutrient status across dietary characteristics

On the current study a positive correlation of iron supplementation with Hb value was seen. This result is in line with a previous study conducted by Dawson & McGanity (1987) where the enrolled participants were less than 16 weeks pregnant women with normal hematologic status that were supplemented with multivitamin/multimineral supplements with or without iron. On the previous study it was also shown that IFA supplements increase serum ferritin, serum iron, and transferrin level. However, on the current study the effect of IFA supplementation on iron, ferritin, transferrin and folate level was not significant. This variation could be because the mothers on the earlier study had normal hematologic condition and the difference after supplementation was assessed. But on the current study almost 60% of the participants were anemic on the time of the study and no intervention was done before sample collection and neither was there adherence assessed.

Although dairy products are rich in calcium and are expected to inhibit iron absorption (Hallberg *et al.*, 1991), a weak positive association (P=0.04) was observed by the current study. An adaptive response through time, possibly involving an up regulation in the efficiency of iron absorption, has been suggested as a possible explanation for the absence of values indicating inhibition of iron absorption (Bendich, 2001). And also the current study and previous studies (Cook & Reddy, 2001; Reddy *et al.*, 2000) have shown increment of iron level in the body by consumption of flesh foods, it might be attributed to the high bioavailability of hem-iron.

A significant negative association was seen between consumption of tea or coffee after meal and serum iron level (P=0.007). Absorption of iron can be significantly decreased by consumption of tannin containing beverages such as tea (Aspuru *et al.*, 2011). This effect can ascribe to the effective sequestration of a proportion of the iron in unabsorbable tannin complexes affecting absorption (Disler *et al.*, 1975).

Table 6: Correlation analysis of dietary habits with micronutrient level

Diet/ Supplement	Hb r/p value	Micronutrient status (r/p value)				
		Iron	Ferritin	transferrin	Folate	Vit B12
Supplement	**0.26/**	0.045/	0.028/	-0.025/	0.06/	0.48/
	0.002*	0.59	0.74	0.77	0.51	0.57
Flesh foods	**0.165/**	-0.099/	0.071/	**0.213/**	0.034/	0.135/
	0.45*	0.232	0.39	**0.01***	0.69	0.104
Dairy products	**0.17/**	-0.091/	0.041/	-0.091/	0.031/	0.034/
	0.04*	0.251	0.621	0.275	0.711	0.686
Ca rich foods	-0.113/	-0.044/	-0.021/	0.027/	0.005/	-0.001/
	0.176	0.599	0.805	0.746	0.95	0.989
Tea or coffee	-0.023/	**-0.22/**	-0.04/	-0.062/	0.054/	-0.028/
	0.782	**0.007***	0.631	0.454	0.517	0.733

147 participants were included

Hb: hemoglobin; Ca: calcium;

Ferritin result is adjusted for inflammation

* Significant correlation at $p<0.05$

4.5 Hemoglobin level and serum micronutrient status of pregnant women

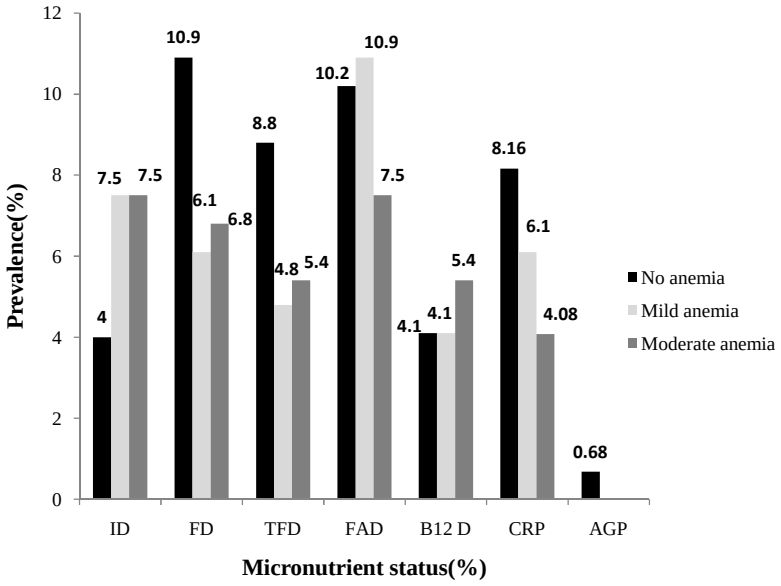
Among the anemic 87(59.2%) pregnant women, 34(23.1%) were mildly anemic with a median (IQR) Hb level of 10.2(0.27)g/dl, 53(36.1%) were moderately anemic with a median (IQR) of 9.6(0.8)g/dl and none of the pregnant mothers were severely anemic as illustrated on **Table 4.5 and 4.6**. From the micronutrients under investigation higher prevalence of ferritin and folate deficiency was observed with a prevalence of 23.5% and 21.8% respectively. Cobalamin deficiency was prevalent in 13.6% of the participants.

Serum inflammatory markers were raised in 18.4% of the mothers with CRP>5mg/L and 0.7% with AGP>1g/L and these values were used to correct serum ferritin level cut off point. According to WHO/CDC cutoff point, FD for the participants with raised CRP and AGP was diagnosed for serum ferritin value of <30ng/ml to adjust for the increase due to inflammation.

Table 7: Hb and serum micronutrient status

Characteristics	N (%)
Anemia based on Hb value	
Anemic (Hb<11g/dl)	87(59.2)
Mild (Hb 9-11g/dl)	34(23.1)
Moderate(Hb 7-9g/dl)	53(36.1)
Non anemic(Hb>11g/dl)	60(40.8)
ID (serum iron<0.33mg/l)	28(19)
IDA (SF<15ng/ml or <30ng/ml)	39(26.5)
Low Serum transferrin>3.1g/l	28(19)
Inflammation (Serum CRP>5mg/l)	27(18.4)
Inflammation (Serum AGP>1g/l)	1(0.7)
FAD (Serum folate<3ng/ml)	32(21.8)
Cobalamin (Serum vit B12<200pg/ml)	20(13.6)

Hb: hemoglobin; ID: iron deficiency; IDA: iron deficiency anemia; SF: serum ferritin; FAD: folic acid deficiency; AGP, α-1 acid glycoprotein; CRP, C-reactive protein; Ferritin result is adjusted for inflammation


12
10
8
6
4
2
0
Prevalence(%)
7.5
7.5
4
10.9
6.1
6.8
8.8
4.8
5.4
10.2
10.9
7.5
4.1
4.1
5.4
8.16
6.1
4.08
0.68
ID
FD
TFD
FAD
B12 D
CRP
AGP
Micronutrient status(%)
No anemia
Mild anemia
Moderate anemia

4.7 Micronutrient status of pregnant women by categories of anemia

The median serum iron and folate levels decreased in anemic pregnant women than non anemic from 0.79mg/l to 0.52mg/l and from 4.8ng/ml to 3.8ng/ml respectively. Among the micronutrients under investigation the major contributors for anemia were iron (19%) and folate (21.8%) deficiency. These findings are comparable with some previous studies which also defined iron and folate being the major predictors for anemia (Chopra *et al.*, 1967; Dilshad *et al.*, 2010; van den Broek *et al.*, 2000).

Median results of cobalamin were comparable among anemic and non anemic group. This result is to some extent comparable with previous studies (Abdelrahim *et al.*, 2008; Gibson *et al*., 2008).

Table 8: **Correlation analysis of micronutrient status for anemic and non anemic pregnant women**

Micronutrient	Anemic		Non anemic		Total		P value
	N%	Median (IQR)	N%	Median (IQR)	N%	Median (IQR)	
Hb(g/dl)	59.2	9.98(1)	40.8	11.9(0.5)	100	10.2(2)	0.00*
Iron(mg/l)		0.52(0.33)		0.79(0.5)		0.59(0.51)	0.00*
ID	15		4.1		19	0.26(0.06)	
Normal	65.4		36.7		81	0.66(0.46)	
Folate(ng/ml)		3.8(2.33)		4.8(2.59)		4.22(2.3)	0.00*
FAD	19.7		2.04		21.8	2.5(0.39)	
Normal	40		38		78.2	4.7(1.9)	
Ferritin(ng/ml)		26.7(37.5)		27.9(31.8)		27.31(31.7)	0.68
IDA	15		11.6		26.7	11.1(2.44)	
Normal	44.3		29.3		23.8	35.3(35)	
transferrin(g/l)		2.5(0.9)		2.5(0.94)		2.46(0.9)	0.51
Elevated	10.2		8.8		19	3.47(0.58)	
Normal	49		32		81	2.32(0.68)	
Cobalamin(pg/ml)		272(112)		267(114)		272.2(115)	0.29
Deficiency	9.5		4.1		13.6	173.5(29.8)	
Normal	59.2		40		86.4	291(100)	
CRP(mg/l)		1.21(2.8)		0.89(3.15)		1.12(2.93)	0.67
Inflammation	10.2		8.2		18.4	7.7(3.6)	
Normal	49		32.7		81.6	0.92(1.51)	
AGP(g/l)		0.32(0.22)		0.34(0.26)		0.33(3.6)	0.23
Inflammation	-		0.7		0.7	1.66	
Normal	59.2		40		99.3	0.33(0.23)	

Hb: hemoglobin; ID: iron deficiency; IDA: iron deficiency anemia; FAD: folic acid deficiency; CRP: C - reactive protein; AGP; α-1-glycoprotien; NS: no significant correlation

Values are expressed as median (IQR); N%: number of pregnant women expressed in percentage; *significant correlation at $p<0.05$

CHAPTER V- CONCLUSION AND RECOMMENDATION

5.1 Conclusion and recommendation

The study showed that the risk of developing anemia has no significant association with socio-demographic or obstetric characteristics of the pregnant mothers. However nutritional characteristics such as IFA supplement and flesh and dairy product consumption were positive predictors of anemia while consumption of tea or coffee after meal negatively affected iron absorption and in turn decreased Hb level. Among the micronutrients assessed serum iron and folate were significantly associated with anemia.

Although IFA supplementation for pregnant mothers is being implemented as a major intervention to decrease the prevalence of anemia, compliance to the supplement and time of initiation should further be assessed to ascertain effectiveness. On the other hand pregnant women should be encouraged to consume flesh foods as they are good source of iron, folate and cobalamin and avoid tannin containing beverages as they have been shown to inhibit iron absorption specially non-heme iron, which is the major source of iron in developing countries, and lead to pregnancy complications and adverse outcomes.

This research was health facility based research where only those who visited the ANC clinics where included which homogenized the characteristics of the participants which in turn could have limited our findings but was still able to find the key associations anticipated. Due to financial reasons, laboratory analysis was done on 147 participants and dietary assessment was not exhaustively assessed. The relatively smaller sample size could also have an effect on representativeness of the data to the whole population. For this reason larger scale research involving larger sample size selected from the community should be conducted for a wider picture.

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COLLEGE OF NATURAL SCIENCES
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Ref: ቁጥር: CNSDO/216/07/15
Date: ቀን: January 13, 2015

To Whom It May Concern

The Ethical Committee of the College of Natural Sciences in its meeting held on 22/12/2015 (Minutes No.12) has examined the project entitled **"Vitamin a, B12, Folate and Hemoglobin Status of Third Trimester Pregnant Women Attending Care"** by Teshome Bekele (Department of Center for food Science and Nutrition) for ethical approval.

The Proposal is approved for implementation. The decision will remain valid until December 21, 2015.

With regards,

Negussie Retta, (Professor)
Dean, College of Natural Sciences

Encl:

- RERC Minutes

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Please quote our reference number in your correspondence.

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

"ሁሉን ነገር መርምሩ፤ መልካሙን ያዙ"

Annex II: SI unit, Conversion factors and alternative units of analyte

Analyte	Normal Range	SI unit	Conversion factor	Alternative Unit
Hemeoglobin concentration		g/L		
Retinol	µmol/L	µmol/L	x 0.286	mg/L
Serum folate		nmol/L	x 0.441	µg/L
Serum vitamin B12		pmol/L	x 1.357	ng/L
Ferritin	15-150 µg/L	µg/L	µg/L x 2.247 = pmol/L µmol/Lx445000=ng/mL µg/L = ng/mL,	pmol/L µmol/L ng/mL=µg/L
Transferrin	2.71-3.91 g/L	g/L	mg/dL x 0.01 = g/L, g/L x 100 = mg/dL, g/L x 12.6 = µmol/L µmol/L x 0.0796 = g/L).	mg/dL µmol/L µmol/L x 0.0796 = g/L).
Serum Folate	8.6-37.0 ng/mL	ng/mL	nmol/L x 0.44 = ng/mL, ng/mL x 2.27 = nmol/L.	nmol/L ng/mL
Serum B12	150-200 pg/mL	pg/mL	pmol/L x 1.36 = pg/mL pg/mL x 0.738 = pmol/L	pmol/L pg/mL
C-reactive protein	<6.81 mg/L	mg/L	mg/L x 9.52 = nmol/L, mg/dL x 95.2 = nmol/L, mg/L x 0.1 = mg/dL, mg/dL x 10 = mg/L, mg/dL x 0.01 = g/L and g/L x 100 = mg/dL.	mg/L nmol/L, mg/dL g/L

Annex III: Consent form (English)

Title: Vitamin A, B12, folate, and hemoglobin status in third trimester pregnant women of West shoa Zone of Oromia Region, Ambo Health Center.

Principal Investigator: Teshome Bekele

Institutions: Food Science and Nutrition, Addis Ababa University

Introduction

Folate has proven to be critical in reducing the risk of neural tube defects. The brain and spinal cord development from the neural tube, and defects in its orderly formation during the early weeks of pregnancy may result in various central nervous system disorders and death. I have decided to study food science and nutrition at Addis Ababa University. This is because my country is among one of the world's food insecure countries and still our people are not aware of nutrition even if nutrition plays a significant role in their life. Most evidences support that many chronic diseases such as heart disease, cancer and cardiovascular diseases are highly linked with poor nutrition and conversely, that consuming nutritious and functional foods can reduce the risk of these diseases. My study is thus an opportunity to investigate the incidence of Iron Deficiency Anemia among pregnant women in with the hope of generating evidenced- based data than can improve the quality of antenatal services offered. Maternal micronutrient nutrition is an important determinant of size and body composition of the fetus. Maternal iron, iodine, calcium, folate, vitamin A, and vitamin C nutrition all influence offspring size.

In Ethiopia, the incidence of malnutrition is relatively very high and also most statistical data revealed that malnutrition is a greatest single cause of child death in the country. Previously, the government has been designing strategies to double production as a sole solution for food insecurity and malnutrition. However, even if there were improvements in the production, there is little reduction in malnutrition. Therefore, my aim as a citizen of Ethiopia is to address the issue of malnutrition and make great contribution to the ambitious plan of the government through research and teaching.

Objective of the Study:

The objectives of this study will be to determine the serum retinol, serum ferritin, serum transferrin, C-protein reaction (CRP), serum B12 and hemoglobin status in 3rd trimester anemic pregnant women. Specifically 1) To evaluate Iron Deficiency in pregnant women, 2) To evaluate Vitamin A deficiency of anemic and non anemic in third trimester pregnant women, 3) To investigate the relationship between anaemia and folate, B12 and vitamin A status of pregnant women and 4) To investigate folate and B12 status of pregnant mothers

Procedures

If you agree to participate, we will conduct the research on blood samples collected from you and will be analyzed in Addis Ababa University or Ethiopian Public Health Institute in addition we will ask you about your food intake pattern, health and information related to your social and demographic characteristics. The interview will only take 10-20 minutes; it will be transported to Addis Ababa for analysis.

Risks: nothing harm full will come to you, as the method only involves analysis of the serum retinol, serum folate, B12 and hemoglobin level of your blood. If you have questions about your rights as a participant of research, you may contact to Mr. Teshome Bekele:(0911543975 or 0942574564) or by E-mail elemabekele@gail.com/eteshomebekele@yahoo.com Or Dr.Kaleab Baye: (0911890489) or by E-mail kaleabbaye@gail.com

Benefits

There are no direct benefits to you. Therefore taking iron supplements before assessing iron status is clearly unwise; hemoglobin tests alone would fail to make the distinction because excess iron accumulates in storage. However the findings will possibly help others. The finds has significant benefits for the understanding of Iron Deficiency Anemia is a major public health problem particularly among pregnant women in developing countries with adverse effects on the mother and the new born it is considered

as the single most prevalent nutritional deficiency. Worldwide my study will aim to assess the problem/limitations of health care practitioners to treat Iron Deficiency Anemia with other micronutrient deficiency. It contribute to the strategic objective of National Nutrition Program to reduce the prevalence of stunting up to 30%; of chronic under nutrition in women of reproductive age to 19% by 2015 by supplementing an appropriate micronutrients for only those deficient in specific micronutrients and Saves the cost of hungry which is estimated about 12% more of Global Development Program for only anemia cases in Ethiopia.

Cost

There is no cost to for participant.

Compensation

There will be no compensation for participating

Participant Rights

If I have said things that are not clear to you, you may ask without hesitation and I will answer. You may feel free and ask questions. Your participation in the study is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. If you don't agree to participate, you can say no without worry. You are not under any obligation to participate in this research project; there are no negative consequences to deciding not to participate your health centre and extension health worker will continue to provide health service to you as usual.

If you do agree to participate, you are not obliged to answer specific questions or to provide information you do not wish to give. You have the right to not answer specific questions but continue as a participant. If you choose to participate and have agreed to have the interviews; yet, you can withdraw from the interview by stating that you have decided to withdraw.

In addition, you can withdraw from the project up until the point when I provide the summary report of the interviews. There will be no negative consequences to withdrawing from the research project. You can state your intention to withdraw from the project by contacting me, Teshome Bekele (the researcher), whose contact information is provided at the end of this form. If you choose to withdraw from the project please indicate whether you want the previously collected data destroyed or returned to you.

Confidentiality

Test result and any information will be kept private .only the research team will have access to your information. When we write a report every ones information will be put together so that information about you or any other individual cannot be seen your blood will be identified with random numbers on serum vials. A list with the names and numbers will be kept in a private, locked file cabinet.

Persons to contact

If you have any questions, you can ask at any time if you have additional question about the study you may contact to Mr. Teshome Bekele at phone number (0911543975) E-mail. elemabekele@gail.com.

Or Dr. Kaleab Baye (0911890489) E-mail kaleabbaye@gail.com

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

Thank you for your cooperation.

Signature:

Mother's name_____

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

I agree to participate in this study.

_____	_____	_____
Signature of the left thumb impression	printed name	Date
_____	_____	_____
Signature of study representative	Printed name	Date
_____	_____	_____

Annex IV: Consent form (Afan Oromo)

Uunkaa Walii galtee

Mata-dure: Qorannoo Haadholii sadarkaa Vitamin A, Vitamin B12, Folic Aciidii fi sadarkaa hiiga irratti argamu yeroo tajaajila hordoffii da'umsaa magaalaa Amboo Buufata Fayyaa Ambootti gaaggefamu.

Qorannoo Gaaggessaan: Tashoomaa Baqqala

Instituutii: Food Science and Nutrition, University Finfinnee

Seensa

Foolik aciidiin rakkoo sammuu daa'immaanii irra gahuu danda'uuf furmaataa gaarii akka ta'e qorannoodhaan mirkanaayeera(neural tube defect). Guddinni sammuu fi ribuu dugdaa dhibee kana irraa hambisuuf baayye gargaara keessattuu ji'oottan jalqabaa ulfaa irratti. Qorannoo kana ademsisuuf kan na kakkaase sababa biyyi kooo addunyaa kana irraa biyoota hanqina midhaaniitiin midhaamte keessaa ishee tokko ta'uu ishii fi sababa hanga ammaatti hubannoon hangami akka midhaan jireenya keenya keessatti miidhaa fidu sirriitti hubachuu dhabuuti.

Akka ragaaleen ibsanitti dhibeen akka onnee, Kaanserii fi k.k.f hanqinna nyaataa waliin hidhannoo cimaa akka qaban ni ibsa. Qorannoon kiyyaa carraa guddaa sababa ka'umsa hanqinna dhiigaa dubartoota ulfaa raga dhugaa fi haqa qabeessa ta'e dhiyeessuun tajaajila haadholii ulfaa sirriitti gargaara. Hanqinna nyaataa keessa Ironii, Ayoodinii, Foolik aciidii, Vitaamin A, Vitamin C kanneen haadhaa fi daa'immaan midhaan.

Itoophiyaatti ka'umsi hanqina nyaataa sadarkaa ol'aanaa irraa akka jiruu fi sababni hanqinna nyaataa du'a daa'immaaniitiif sababa tokkoofi tokko ta'e qofaa dha. Kanaan dura mootummaan istraateejiin du'a haadholii hir'isuu baayyee hojii irra oolchaa jira. Haa ta'uu malee carraaqiin kun hanga ta'e hir'isaa jiraatulle hanga ammaatti miidhaan hanqina nyaata ammas guutummaa guutuutti furmaata hin arganne. Kanaafuu, kaayyoon koo akka dhalattaa biyyaatti dhimma hanqinna nyaataa kana hir'isuuf tattaafatuu fi

karoora mootummaan dhimma kana irratti baasegalmaan ga'uus gahee koo qorannoo fi barsiisuun ni baha.

Haala Qoranichaa

Yoo ati qorannoo kana irratti himaachuuf fedhintaa kee nuu ibsite dhiiga qorannoo kanaaf ta'u sirraa fudhachuun laboratoorii keessatti qorannoon ni adeemsifama. Gama biraatiin odeeffannoo waa'ee haala sirna nyaataa keessaanii yoo guddatee daqiiqaa 15 hanga 25tiif afaaniin isin gaafachuun sababoota dhibee kanaaf ta';uu danda'an jedhamanii yaadaman qorachuuf ni gargaara.

Miidhaa

Miidhaa ilaalchisee midhaa tokkolle sirratti hin geessisu, jechuun takkaa dhiigni keessan fudhatamnaan laboratoorii keessatti adeemsifama malee wanti isin wajjiin walitti fidu tokkolle hin jiru. Gaaffilee adda addaa yoo isinti uumamte bilbila kootiin 09111543975 ykn 0942574564 imeeliin koo elemabekele@gail.com/eteshomebekele@yahoo.com Karaa biraatiin Dr. Kaleab Baye (09111890489) imeeliin isaanii immoo kaleabbaye@gail.com gaafachuun ni danda'ama.

Bu'aa:

Bu'aan kallattidhaan argamuu hin jiru. Wanti beekkamuu qabuu yeroo ulfaa keessatti atoo sababa ka'umsa dhibee hanqina dhiigaa kun dhufeen hin beekin ykn qorannoo dhiigaa hanqinna Ironii qofaan murteeffamuun Ironii fudhachuun sirrii miti maliif jennaan yoo Ironii baay'inaa nafa keessa jiraate dhibeen haadha fi daa'immaan irra gahu cimaa waan ta'eef. Kana jechuun qorannoon kun alkallaattiidhaan sababa ka'umsa dhibee kanaa ni ibsa, biyyoota guddataa jirani irratti sababni dhibee kanaa maal akka ta'e beekuuf carraa ni uuma. Addunyaaf immoo beektonnii dhibee kana wal'aanuuf carraaqan bu'aa baayye irraa argatu. Karaa biraatiin sababa qancara daa'immaaniitii fi baasii Itoophiyaa keessatti qofa waa'ee beelaatiin sagantaa guddinaa Walii gala iraa 12% ba'u ni hir'isa.

Baassii

Baasiin namni qorannoo kana irratti hirmaatu baasu tokkollee hin jiru.

Bakka Bu'ummaa

Sababa dhiigni keessan qorannoof fudhatameen bakka bu'ummaan wanti kaffalamu hin jiru.

Mirga Hirmaattotaa

Wanti gaaffii uumu yoo jiraate sodaa fi shakkii tokko malee akka na gaafattan isin ni jajjabeessa gaaffii keessan hundaaf deebiin ni kennama. Hirmaachuuf murteessuun mirga keessan keessan qofa. Yoo fedhintaa hin qabaanne hin ta'u jechuun deebii kennuu ni dandeessu. Ogeessi fayyaa fi Exteenshiiniin fayyaa buufata fayyaa keessanii tajaajila barbaachisu hundumaa isiniif ni laatu.

Mirkanneessan keessan

Qorannoo fi deebiin/firiin qorannoo keessanii garee qoranichaa adeemsisuu (buufata qorannoo)f malee qaama biraatiif dabarfamee hin kennamu. Qoranichi erga adeemsifamee bu'aan argamee booda gabaasni yoo dhiyaatu walitti cuunfamee waan dhiyaatuuf qabxiin eenyuu kan eenyu akka ta'e hin beekkamu kana jechuun firii qorannoo nama dhunfaa namni biraa beekuu hin danda'u. Koodiin kennameefii laakkofsa qofaan galmee keessatti hidhamee taa'a.

Qaamni Dhimmichi ilaallatu

Akka carraa wanti gaaffii uumu yoo jiraate yeroo barbaaddanittin gaafachuu ni dandeessu. Obboo Teshoomee BeqqeleeLakk. Bilbilaa (0911543975) E-mail. elemabekele@gail.com.

Or Dr. Kaleab Baye Lakk. Bilbilaa (0911890489) E-mail kaleabbaye@gail.com

Yoo qorannoo kana irratti hirmaachuuf fedhintaa qabaattan maqaa fi mallaattoo keessanii asii gaditti mirkaneessa.

Qorannoo kanairratti waan hirmaattaniif galatooma

Mallattoo

Maqaa Haadhaa_____

Qoranichi naa ibsamee ifa naa ta’eera. Qorannoo kanairratti hirmaachuuf waliigaleera.

_____	_____	_____
Mallattoo	Maqaa barreeffamaan	Guyyaa
_____	_____	_____
Mallattoo bakka bu’aa qoranichaa	Maqaa barreeffamaan	Guyyaa

Annex V: English questionnaire

	A questionnaire for the Nutritional Assessment and practices of second and third trimester pregnant Women			
Hello, my name is _____. I am a student of Food Science and Nutrition in Addis Ababa University. I am interested in gathering information about the opinions and practices of pregnant women with iron-deficient anemia. This information is important for elaborating strategies to improve quality of care provided at prenatal clinics. Your participation in this survey will contribute to this purpose. The information you are going to provide will be used only in scientific purposes, your name will remain confidential. The interview will take 15 minutes.				
Do you agree to be interviewed? A. Yes B. No Thank you, let's start.				
A. Socio-demographic, economic and lifestyle characteristics				Code_____
1.0 Personal information				Address_____
Name (Optional)_____				
1.1	Age_____Height_____Cm			
1.2	Occupation:	A. Farmer	B. Housewife	C. Merchant
1.3	What is your monthly income?	_____		
1.4	What is your educational status?	A. literature	B. Read & write	C. Educated
	If Educated	A.1^0school	B.2^0school	C. 3^0/University
1.5	What is your marital status?	A. Married	B. Single	C. Other
1.6	What is your religion?	A. Christian	B. Muslim	C. Other
2.0	The nature of complications during pregnancy			
2.1	How many children do you have?		_____	
2.2	Where did you deliver your last babies?		A. Healt institute	B. Home
2.3	Do you have source of information about nutrition in pregnancy?		A. Yes	B. No
2.4	If yes, what is the source of your information?		A. Radio/Televion	B. Newsletter/Internet

			C. Community advocacy/H.extension worker	
			D. School	E. Other, Please specify
2.5	Was there any blood loss in your previous delivery?		A. Yes	B. No
2.6	Did you follow antenatal care in your previous pregnancy?		A. Yes	B. No
2.7	What there any blood loss in your current pregnancy?		A. Yes	B. No
2.8	Is it your first pregnancy?		A. Yes	B. No
2.9	At what interval did you deliver your babies?		A. < 2 yrs	B>2 yrs
2.10	was there any abortion in your pregnancy?		A. Yes	B. No
2.11	Do you use contraceptive?		A. Yes	B. No
2.12	Did you become infected with malaria for the last one year?		A. Yes	B. No
2.13	Do you have anti-malaria bed net?		A. Yes	B. No
2.14	If you say yes for question 2.18 do you use frequently?		A. Yes	B. No
2.15	Did you have nausea / vomiting at the beginning of the pregnancy?		A. Yes	B. No
2.16	Have you taken iron supplement the current pregnancy?		A. Yes	B. No
2.17	If yes, what kind of supplementation?		1. Iron sup	3. Anti-acid
			2. IFA	4.Other (specify) _____
2.18	Notice any changes in your appetite since you became pregnant?		A. Yes	B. No
2.19	If yes, specify (please note any increase/decrease)		Increase	Decrease
2.20	Do (did) you experience some symptoms like:	A. Persistent swelling of feet, hands	B.Increasing breathless, especially on routine activity	C. Headaches

		or face		
		D. Blurring of Vision	E. Fever) temperature > 38	F. High colored urine in the past two weeks.
3.0	Type of foods/Twenty four hour recall food questionnaire			
3.1	Number of meals per day?___	1/day	2/day	3/day
3.2	Did you eat the following foods in the past two weeks?		A. Yes	B. No
3.3	Iron rich foods such as, Meat and meat products, eggs, bread, green leafy vegetables, pulses and fruits		A. Yes	B. No
3.4	Animal source foods such as, milk, yogurt and cheese		A. Yes	B. No
3.5	Calcium rich foods such as, dairy products, cabbage, eggs and		A. Yes	B. No
3.6	Did you practice any fasting since you became pregnant?		A. Yes	B. No
3.7	When do you fast?	A. Per month	B. Per year	C. per weak
3.8	For the question number 3.5 how many times?	A. 1	B.2	D.4
			C.3	E.>4
3.9	If per day only		A. Only meat	B<6hrs
			C1/2 day	D. Full day
3.10	Level of fasting?	Deprivation of animal source food except fish		Deprivation of all animal source food
3.11		Deprivation of animal source food + no breakfast		Deprivation of animal source food + fasting until 3.00 Pm
3.12	Did you drink tea/coffee daily in the past two weeks?		A. Yes	B. No
3.13	If yes, how many cups did you drink on average per day?		_____	

Annex VI: Afan Oromo questionair

	Gaaffii fi Deebii Afaanii gulaalli Muuxannoo fi Sirna Nyaataa Haadholii mana yaalaa Hordofanii yeroo da'uumsa I assniitti			
Akkam Jirtu, Ani Maqaan koo _____jedhamaa barataa Yuniversiitiii Finfinnee sagantaa Nyaataa fi Sirna nyaataa irraa. Waa'ee muuxannoo fi carraalee mala nyaataa irratti odeeffannoo haadholii ulfaa kan hanqinna dhiigaa qabanii walitii guuruuf gammachuun guddaan natti dhaga'ama. Odeeffannoon kun istraateejii qulqullinna haadholii ulfaatiif kennamu irratti gar malee faayidaa qabeessa. Kunimmoo kan galma gahuu danda'uun hirmaannaa keessan qofaan. Odeeffannoon isin nuu kennitan kan ta'u tajaajila qorannoo qofaaf yoo ta'u maqaan keessaan icciitiidhaan qabama eenyulle beekuu hin danda'u. Gaaffiif deebiin kun yoo guddate daqiiqaa 15 hanga 20tii				
Gaaffiif deebii kana itti fufuuf fedhintaa qabduu? A. Eeyyeen B. MIti Galatoomaa itti fufuu ni dandeenya				
A. dhuunfaa Ilaalchisesee gaaffii gaafatamu				Mallttoo/Koodii
1.0 Odeeffannoo dhuunfaa				Address_____
Maqaa (Dirqamaa miti)_____ _____				
1.1	Umrii_____Dheerinna___Cm			
1.2	Hojii:	A. Q/Bultuu	B. Ha/manaa	C. Daldaltuu
1.3	Galii dhunfaa ji'aan?	_____		
1.4	Sadarkaa barumsaa keessan?	A. Hibaratin	B. Bar fi Dub	C. kan baratte
	Yo kan baratte ta'e	A. Sad 1ffaa	B. Sad 2ffaa	C. Koll/Yuniversiti
1.5	Haala maatii?	A. heerumte	B.hin heerumne	C. kan biraa
1.6	Amantaa?	A. Kiristaana	B. Musliima	C. kan biraa
2.0	Haalawwan yeroo ulfaa kana doorsisan			
2.1	Kanaan dura ijoollee keessan eessatti deessan?		A. Buf fayyaa	B. Manatti

2.2	Odeeffannoo mala nyaataa kanaan dura qabduu?		A. Yes	B. No
2.3	Maddii odeeffannoo nyaataa keessan hoo?		A. Raadoo/TV	B.Gaazexa/Interneet ii
			C. Exteenshiinii fayyaa	
			D. M/barumsaa	E. kan biraa yo ta'e
2.4	Dhiigaa kanaan dura mudatee turee?		A. Yes	B. No
2.5	Ulfa kana irraatti dhiigni jige hoo jiraa?		A. Yes	B. No
2.6	Ulfaa yeroo jalqabaa keessan moo??		A. Yes	B. No
2.7	Garaagarummaan ijoollee keessan itti deessan meeqa?		A. < 2 yrs	B>2 yrs
2.8	Kanaan dura ulf isinirraa bahe jiraa moo?		A. Yes	B. No
2.9	Karoora matiitti fayyadamtanii turtan moo?		A. Yes	B. No
2.10	Waggaa tokkoon darbee keessatti dhibee dhukkuba buusa qabduu?		A. Yes	B. No
2.11	Marabii dhibee bookee hir'isu qabduu?		A. Yes	B. No
2.12	Gaaffii 2.18 eeyyen yoo ta'e yeroo hunda fayyadamtu moo?		A. Yes	B. No
2.13	Nyaanni isin deddeebisuun yeroo jalqabaa ture moo?		A. Yes	B. No
2.14	Qorichii biraa ulfa kana irratti fayyadmatan jira moo?		A. Yes	B. No
2.15	Yoo jiraate gosa isaa addaan haabaasanu		1. Iron sup	3. Anti-acid
			2. IFA	4.Other (specify) _____
2.16	Fedhintaa nyaataa keessan irratti dhiibbaan fide jiraa?		A. Yes	B. No
2.17	Yoo jiraatee hir'ataa deeme moo dabataan		Increase	Decrease
2.18	Mallaattoo dhibee asii gadiitiin akka tasaa qabamtanii jirtan moo?	A. Persistent swelling of feet, hands or face	B.Increasing breathless, especially on routine activity	C. Headaches
		D. Blurring	E. Fever)	F. High colored

		of Vision	temperature > 38	urine in the past two weeks.
3.0	Gaaffilee midhaan nyaataa yeroo gabaabaa keessatti argatan qabachiisuuf			
3.1	Lakkoofta baay'ina nyaataa argatan	1/day	2/day	3/day
3.2	Gosa nyaataa asii gaditti ibsame kana keessa yoo jiraate?		A. Yes	B. No
3.3	Ironii dhaan guutamaa kan ta'a kan akka Foonii, Raafu, hanqaaquu, koolaa fi fuduraa fi kudraa adda addaa		A. Yes	B. No
3.4	Nyaataa loonii keessaammoo aannan, dhadhaa fi itittuu?		A. Yes	B. No
3.5	Calcium dhaan kan guutaman hoo kan akka aannanii, itittuu		A. Yes	B. No
3.6	Ulfa kana irraatti xoomiin adda adddaa jira turee?		A. Yes	B. No
3.7	Yeroo akkamii soomtu?	A. Per month	B. Per year	C. per weak
3.8	Deebii armaan olitti deebistaniif yeroo meeqatti?	A. 1	B.2	D.4
			C.3	E.>4
3.9	Guyyaatti yoo ta'e akka armaan gadiiitti haa ibsan?		A. Only meat	B<6hrs
			C1/2 day	D. Full day
3.10	Sadarkaan xoomii keessani hoo maal fakkaata?	Deprivation of animal source food except fish		Deprivation of all animal source food
		Deprivation of animal source food + no breakfast		Deprivation of animal source food + fasting until 3.00 Pm
3.11	Shaayii fi buna hoo ni dhugduu?		A. Yes	B. No
3.12	Yoo dhugdan ta'e yero akamiitti akka dhugdan tarreess?		_____	

Food science and Nutrition center

College of Natural Science

Addis Ababa University

RESEARCH THESIS APPROVAL

This is to certify that the thesis prepared by Lewam Mebratu, entitled*: Identification of risk factors for anemia on third trimester pregnant women at West Showa Zone, Ambo, Ethiopia* and submitted in partial fulfillment of requirements for the degree of Master of Science in food science and nutrition complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

……	……	…..…….
Name of chairperson	Signature	Date
……	……	…..…….
Name of advisor	Signature	Date
……	……	…..…….
Name of internal examiner	Signature	Date
……	……	…..…….
Name of external examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the College of Graduate Studies (CGS) of the candidate's major department.

I hereby certify that I have read this thesis prepared under my direction and recommend that it is accepted as fulfilling the thesis requirement.

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Chair of Department or Graduate program Coordinator