



Anemia among Pregnant Women in Kelela Health Center, South Wollo, Northeast
Ethiopia

By
Aragaw Mohammed

A Thesis Submitted To Department of Zoological Sciences, In Partial Fulfillment of the
Requirements For the Degree of Master of Science In Biology

Supervisor: Hassen Mamo (PhD)

September 2016
Addis Ababa
Ethiopia

Table of Contents

Content	page
Table of contents.....	ii
Acknowledgments.....	iv
Acronyms.....	v
List of tables.....	vi
List of figures.....	vii
List of annexes.....	viii
Abstract.....	xi
1. Introduction.....	1
2. Objective.....	3
2.1 General objective.....	3
2.2 Specific objectives.....	3
3. Literature review.....	4
3.1 Types and determinants of anemia.....	4
3.1.1 Micronutrients as causes of anemia.....	5
3.1.2 Infections and chronic illnesses.....	6
3.2 Pregnancy anemia.....	7
3.3 Anemia treatment and prevention.....	8
4. Materials and Methods.....	12
4.1 Study area.....	12
4.2 Study design and population.....	12
4.3 Data collection procedure.....	12
4.3.1 Questionnaire.....	12
4.3.2 Hemoglobin determination.....	14
4.4 Data analysis.....	14
4.5 Ethical considerations.....	14
4.6 Data quality control.....	14
5. Results.....	15
5.1 Study population.....	15
5.2 Anemia prevalence and risk factors.....	15

Content	page
6. Discussion.....	19
7. Conclusion and recommendation.....	22
8. References.....	23
9. Annexes.....	28
10. Declaration.....	36
11. Statement of supervisor(s).....	37

Acknowledgments

My heartfelt thanks go to my advisor Dr Hassen Mamo for his valuable advice and close follow up in preparing this research paper. I would like to thank Addis Ababa University School of Graduate studies for its financial support. I am grateful to Kelela Health Center staffs and the study participants for their kind cooperation. Also, I would like to extend thanks to all of my colleagues and family for their support in my academic life. Last but not least thanks go to my wife Fozya Tadesse for her support in my social and educational life.

Acronyms

ANC	Antenatal Care
AOR	Adjusted Odd Ratios
CI	Confidence Interval
COR	Crud Odd Ratio
CSA	Central Statistical Agency
HA	Hemolytic anemia
Hb	Hemoglobin
IDA	Iron Deficiency Anemia
KHC	Kelela Health Center
RBCs	Red Blood Cells
STD	Standard for hemoglobin
WHO	World Health Organization

List of Tables

Table	page
Table 1 Univariate logistic regression analysis of socio-demographic, clinical and dietary variables in pregnant women attending ANC follow up at Kelela health (KHC), January-May 2016 (N=243)	16
Table 2 Multivariate logistic regression analysis of anemia by socio-demographic, clinical feature and dietary habit determinants in pregnant women attending ANC at KHC, January-may 2016 (N=243).....	17

List of Figures

Figure	Page
Fig 1 Map of Kelela woreda.....	13
Figure 2 Over all prevalence of anemia among pregnant women attending ANC at Kelela health center and different parts of Ethiopia.....	18

List of Annexes

Annex	Page
Annex 1 Consent form.....	28
Annex 2 Amharic version of the consent form.....	29
Annex 3 Questionnaire.....	30
Annex 4 Amharic version questionnaire	31
Annex 5 Procedures of hemoglobine estimation by sahli's-healing method (sahli's hemoglobino meter).....	32
Annex 6 Picture of sample collection and facilities of the laboratory in KHC.....	34
Annex 7 Ethical clearance	35

Abstract

Anemia is a significant health challenge in both low- and high-income countries with major consequences in public health as well as socio-economic development. It is regarded as a major risk factor for unfavorable outcome of pregnancy both for the mother and the fetus. Current setting-specific information on this formidable threat is essential to design and implement appropriate control interventions. However, there is lack of adequate information on the magnitude of anemia among pregnant women in Kelela, northeast Ethiopia. This study was, therefore, done to determine the prevalence of anemia and associated factors among pregnant women attending Kelela health center (KHC). A cross-sectional study was carried out among pregnant women who came for their antenatal care follow-up between January and May 2016. A pretested questionnaire was used to gather socio-economic, demographic and clinical characteristics of the participants. Hemoglobin (Hb) was measured using the Sahli-Helling method and anemia status determined ($Hb < 11.0 \text{g/dl}$). Univariate and multivariate logistic regression analysis were used to test the association between anemia and socio-demographic variables ($p < 0.05$). The prevalence of anemia was 58.8% ($n=243$); mild 40.8%, moderate 59.8%, and no severe cases. Women having birth interval of < 2 years were twice likely to be anemic than those with ≥ 2 years birth interval (adjusted odds ratio (AOR) 2.235, 95% confidence interval (CI) 8.427-8.677, $p = 0.001$). Moreover, anemia was significantly higher among the multigravidae (AOR 0.212, 95% CI 0.048-0.941, $p = 0.041$) and those in their third trimester of pregnancy (AOR 6.582, 95% CI 1.851-23.401, $p = 0.004$). The prevalence of anemia in the study was high, although there were no severe cases, calling for immediate intervention.

Keywords: Anemia, pregnancy, prevalence, maternal, KHC

1. Introduction

Anemia is the most serious blood disorder characterized by abnormal red blood cells (RBC) that affect the normal distribution of oxygen to the different body parts. The problem with the RBC may involve the hemoglobin (Hb), cell membrane, or enzymes that maintain healthy RBC. The RBCs distribute oxygen to body tissues through their Hb molecule. Thus, anemia actually refers to the deficiency or abnormality of Hb synthesis. This in turn has multiple clinical implications and manifestations.

Anemia is a global public health problem with major consequences for human health as well as socioeconomic development. It is the most common disorder of the blood affecting more than 2 billion people globally, accounting for over 30% of the world's population (WHO 2015). Anemia is the most common public health problem in all types of people particularly in low-income countries. Pregnant women and young children carry the highest burden (Rodak and Bernadette 2007).

An estimated 58.27 million women worldwide are anemic during pregnancy, 95.7% of whom live in low-income countries (Van den Broek et al. 2000). In Africa, the prevalence of anemia in pregnancy is estimated to be between 35% and 75% as compared to the developed countries where prevalence is at 4-15% (Van den Broek et al. 2000). Anemia during pregnancy is of great concern because it contributes significantly to increased risk of maternal death during the prenatal period or low-birth weight, or premature birth (<http://americanpregnancy.org>). Further, anemia increases the risk of postpartum hemorrhage (Vos et al. 2012) pregnancy-induced hypertension, placenta praevia and cardiac failure (De Benoist et al. 2008, Beaton 2000, Poogi 2013). Overall, 20-40% of the estimated 50 000 maternal deaths worldwide associated with child birth or the postpartum period are attributed to anemia during pregnancy. Anemia is also an established risk factor for intrauterine growth retardation and subsequent low-birth weight, preterm delivery and prenatal death (Nassreen et al. 2010).

Like any other sub-Saharan African country, anemia is a significant public health problem in Ethiopia. According to the 2007 Ethiopian Central Statistical Agency (CSA) report (WHO 2008)

nationally, 44% of children aged 6-59 months were anemic, with 21%, 20% and 3% having mild, moderate and severe anemia. Moreover, 17% of women aged 15-49 were anemic; of which 13% had mild anemia, 3% moderately anemic, and less than 1% severely anemic. Some other studies in Ethiopia have also reported varying magnitude of anemia and identified associated factors (Melku et al. 2014). Prevalence rates as high as 40.5% in the general population and 47.2% in children were reported from northwest Ethiopia (WHO 1992). Higher rates about 57% have also been reported in pregnant women in southwest Jimma (Desalegn 1993).

Determination of the magnitude of anemia among pregnant women helps to monitor health of the pregnant women contributing to reduction in maternal morbidity and mortality. Also, periodic assessment of factors pre-disposing to anemia in a local area enables to take targeted intervention activities. Therefore, the aim of this study was to assess the current status of anemia among pregnant women attending antenatal care at Kelela town in south Wollo, northeast Ethiopia.

2. Objectives

2.1 General objective

The general objective of this investigation was to assess the prevalence of anemia among pregnant women attending antenatal care (ANC) at Kelela health center (KHC) in south wollo zone northeast Ethiopia.

2.2 Specific objectives

1. To determine Hb levels of pregnant women attending ANC at KHC.
2. To document the prevalence of socio-demographic and other established anemia risk factors among pregnant women at KHC
3. To find the level of association between anemia and its risk factor in pregnant women.

3. Literature review

The World Health Organization (WHO) defines anemia as Hb level below 11g/dl for adult non-pregnant women and 10.5g/dl as the lower limit acceptable in the second-half of pregnancy (WHO 2015). Anemia can further be classified into mild anemia (10-10.9g/dl), moderate (7-9.9g/dl) and severe (<7g/dl). Anemia is common in pregnancy because a woman needs to have enough RBC to carry oxygen around her body as well as to her baby (WHO 2015).

3.1 Types and determinants of anemia

Anemic conditions can result due to blood loss, and decreased production or increased breakdown of the RBC. Blood loss is the most common cause of anemia. Heavy menstrual periods or bleeding in the digestive or urinary tract can cause blood loss. Surgery, trauma, or cancer also can cause blood loss. If a lot of blood is lost, the body may lose enough red blood cells to cause anemia (www.mayoclinic/iron-deficiency-anemia). Causes of decreased RBC production include micronutrient deficiency, thalassemia and a number of neoplasms of the bone marrow among others.

Similarly, a number of genetic conditions such as sickle cell sickness, infections like malaria and some autoimmune diseases, among others, can cause increased RBC breakdown. Excessive destruction of RBCs leads to hemolytic anemia. Hemolytic anemia is a condition in which RBC are destroyed and removed from the bloodstream before their normal lifespan is up. A number of diseases, conditions and factors can cause the body to destroy its RBC. Hemolytic anemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure (Sewpersad and Inkosa 2014). Normally an RBC lives 120 days. As the duration shortens the bone marrow becomes incapable of keeping up with the body's needs, this leads to anemia.

Apart from RBC quantity, anemia can emanate based on the size of RBC which affects the amount of Hb in each cell. If the cells are smaller or larger than the standard two types of anemia occur, microcytic anemia and macrocytic anemia respectively.

3.1.1 Micronutrients as causes of anemia

Most of the anemia are due to inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid (reviewed in Ayenew et al 2014). The most common form of anemia iron deficiency anemia (IDA) which is usually chronic blood loss caused by excessive menstruation. Increased demands for iron, such as foetal growth in pregnancy, and children undergoing rapid growth spurts in infancy and adolescence, can also cause IDA. This condition is treated with iron supplementation as well as the treatment of the underlying cause of the iron deficiency (Vos et al. 2012).

IDA affects nearly 1 billion people globally, accounting for over 15% of the world's population (Vos et al. 2012). In 2013 anemia due to iron deficiency resulted in about 183,000 deaths - down from 213,000 deaths in 1990 (Vos et al. 2012). It is more common in females than males, among children, during pregnancy, and in the elderly (Janz et al 2013). Anemia increases costs of medical care and lowers a person's productivity through a decreased ability to work (Janz et al 2013). In 2002, IDA was considered to be among the most important contributing factors to the global burden of disease. This is the leading cause of anemia in the world, and consequently, the most common type of anemia during pregnancy. It is generally assumed that 50% of the cases of anemia are due to iron deficiency (WHO 2002), but the proportion may vary among population groups and in different areas according to the local conditions. The main risk factors for IDA include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high (i.e. growth and pregnancy) ([www.wbdl.com/diagnosis of anemia](http://www.wbdl.com/diagnosis_of_anemia)).

A diet that lacks iron, folic acid (folate), or vitamin B12 can prevent your body from making enough RBC. The body also needs small amounts of vitamin C, riboflavin, and copper to make RBC. Conditions that make it hard for the body to absorb nutrients also can prevent it from making enough RBC (Sewpersad and Inkosi 2010). In addition to iron, the body needs folate and vitamin B12 to produce enough healthy RBC. A diet lacking in these and other key nutrients can

cause decreased RBC production. Both vitamin B12 deficiency and folate deficiency are more common in older people, affecting around 1 in 10 people above the age of 75 (WHO 2015).

Additionally, some people may consume enough B12, but their bodies aren't able to process the vitamin. This can lead to vitamin deficiency anemia, also known as pernicious anemia (WHO 2015). This is an inherited condition where patients find it difficult to use the vitamin B12 in diet for production of healthy RBCs. This is a rare condition and affects 1 in 10,000 people in northern Europe (WHO 2015).

3.1.2 Infections and chronic illnesses

Infections or chronic illnesses are mostly responsible to induce anemia due to early destruction of the RBC. The type of anemia which results due to the above causes is hemolytic anemia (HA). A number of diseases, conditions, and factors can cause the body to destroy its young RBC. These causes can be inherited or acquired. Sometimes, the cause of HA isn't known. Inherited HA include sickle cell anemia, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and pyruvate-kinase deficiency. Acquired HA include immune HA, autoimmune HA, alloimmune HA, drug-induced HA, mechanical HA, and paroxysmal nocturnal haemoglobinuria (Sukrat et al 2010). Certain infections and substances can also damage RBC and lead to HA.

In inherited HAs, the genes that control how RBCs are made are faulty. Different types of faulty genes account for the different types of inherited HA. In each type of inherited HA, the body makes abnormal RBC, fails to synthesize enough Hb, has deformed cell membrane, or enzymes that maintain healthy RBC are deficient. In acquired HA, the body makes normal RBCs; however, some disease, condition, or factor destroys the cells too early. Examples include immune disorders, infections and reactions to medicines or blood transfusions (Sukrat et al 2010).

An increasing number of studies of helminthes epidemiology have shown that it is common for individuals to be infected with more than one species of helminthes (Brooker 2000) in concurrent

intestinal nematode and schistosome infections. A number of epidemiological studies have indicated that individuals infected with multiple species of helminthes often harbor heavier infections than individuals infected with a single helminthes species (Brooker 2000, Booth 1998). An important consequence of simultaneous infection with the parasites that cause hookworm, schistosomiasis, and malaria is severe anemia (Brooker 2000). The tapeworm *Diphyllobothrium latum* is associated with this type of anemia. Other some types of infections, blood diseases, autoimmune disorders, exposure to toxic chemicals and some medications can also result in a decreased number of RBC and cause anemia.

3.2 Pregnancy anemia

The greatest burden of anemia is born by Asia and Africa where it is estimated that 60% and 52% of pregnant women, respectively recorded in anemic and between 1% and 5% are severely anemic in Asia and Africa respectively and is associated with women of age less than 20 years, third trimester of pregnancy, rural residents, and multiparous women (Jufar and Zewde 2014). United nation expert panel considered severe anemia an associated cause in up to half of the maternal death worldwide. Estimates of maternal mortality resulting from anemia range from 34/100,000 live births in Nigeria to as high as 194/100,000 in Pakistan. In sub-Saharan Africa it is estimated that 20% of maternal death are associated with anemia (Jufar and Zewde 2014).

Anemia increases perinatal risks for mothers and neonates; and increases overall infant mortality. The odds for fetal growth restriction and low birth weight are tripled. The odds for preterm delivery are more than doubled. Even a moderate hemorrhage in an anemic pregnant woman can be fatal. Effects of anemia on fetus and neonate a basic principle of fetal/neonatal iron biology is that iron is prioritized to RBC at the expense of other tissues, including brain. When iron supply does not meet iron demand, the fetal brain may be at risk even if the infant is not anemic. Although dietary deficiency may be contributory, the etiology of the vast majority of cases of IDA in infancy and childhood is maternal iron deficiency anemia in pregnancy (Vilanova 1994).

Anemia adversely affects cognitive performance, behavior and physical growth of infants, preschool and school-aged children. Anemia depresses the immune status and increases the

morbidity from infections in all age groups. It adversely impacts the use of energy sources by muscles and thus the physical capacity and work performance of adolescents and adults (Vilanova 1994).

Pregnancy is a period of a significant increase in iron requirement over and above the non-pregnant state (Zavaleta et al. 2000). Although iron requirements are reduced in the first trimester because of the absence of menstruation, they rise steadily thereafter from approximately 0.8 mg per day in the first month to approximately 10 mg per day during the last 6 weeks of pregnancy (Bothwell 2000). The increased iron requirement is due to expansion of maternal red blood cell mass for increased oxygen transport, including transfer of iron, to both the growing fetus and the placental structures, and as a needed reserve for blood loss and lochia at parturition (De Benoist et al. 2008, Poogi 2013). Lochia is a vaginal discharge after giving birth (puerperium) containing blood, mucus and uterine tissue. Lochia discharge typically continues for 4 to 6 weeks after child birth, which is known as the postpartum period (Oppenheimer et al. 1986) It sterile for the first 2 to 3 days, but not by third or fourth day, as the uterus begins to be colonized by vaginal commensals such as non-hemolytic *streptococci* and *E. coli*. Due to increased iron requirements, pregnancy is also a period of increased risk for anemia. Thus, a high proportion of women become anemic during pregnancy (Oppenheimer et al. 1986).

3.3 Anemia treatment and prevention

To effectively control preventable anemia early and accurate diagnosis is necessary. Both clinical and laboratory diagnosis is possible.

Clinically, when anemia comes on slowly the symptoms are often vague and may include feeling tired, weakness, shortness of breath or a poor ability to exercise. Anemia that comes on quickly often has greater symptoms which may include confusion, feeling like one is going to pass out, loss of consciousness, or increased thirst. Anemia must be significant before a person becomes noticeably pale. Additional symptoms may occur depending on the underlying cause.

The prevalence of anemia in a population is best determined by using a reliable method of measuring Hb concentration (Vilanova 1994). Compared with the cost and difficulty of biochemically assessing the prevalence of iodine deficiency and vitamin A deficiency, the determination of the prevalence of anemia in a population is relatively simple and inexpensive. The only methods generally recommended for use in surveys to determine the population prevalence of anemia by haemoglobinometry are the cyanmethemoglobin method in the laboratory and the HemoCue system.

The cyanmethemoglobin method for determining Hb concentration is the best laboratory method for the quantitative determination of Hb. It serves as a reference for comparison and standardization of other methods (Vilanova 1994). A fixed quantity of blood is diluted with a reagent (Drabkin's solution) and Hb concentration is determined after a fixed time interval in an accurate, well-calibrated photometer.

The HemoCue system is a reliable quantitative method for determining Hb concentrations in field surveys, (Van Schenck et al. 1986) based on the cyanmethemoglobin method. The HemoCue system consists of a portable, battery-operated photometer and a supply of treated disposable cuvettes in which blood is collected. The system is uniquely suited to rapid field surveys because the one-step blood collection and Hb determination do not require the addition of liquid reagents. Survey field staff without specialized laboratory training has been successfully trained to use this device. The HemoCue system gives satisfactory accuracy and precision when evaluated against standard laboratory methods (Johns and Lewis 1989). The packed cell volume is used as a simple screening test for anemia, as a reference method for calibrating automated blood count systems and as a rough guide to the accuracy of hemoglobin measurements (WHO 2015). The microhaematocrit method has an adequate level of accuracy and precision for clinical utility (WHO 2002). Hemoglobin content of blood in solutions may be estimated by several methods, by measurement of its color, erythrocyte, volume fraction, specific gravity or its iron content. The methods are color or light intensity matching techniques that measure inert pigments in blood with different degree of efficiency (Balasubramaniam and Malathi 1992). The type of method is selected based on its feasibility, cost effectiveness,

simplicity, reliability, and easy to use in the laboratory and in the field (Millis and Meadowst 1998). Type of method and site of blood sample used is found to make a significant within subject variability of hemoglobin concentration (Balasubramaniam and Malathi 1992, Mills and Meadowst 1998).

Sahli's method of hemoglobin estimation is relatively inexpensive, simple to use, does not require electricity and requires only small sample of blood. In developing country like Ethiopia most common method used for hemoglobin estimation is the Sahli's method. Keeping in account high cost, and lack of facilities in the developing countries the use of sophisticated equipments is limited for screening of hemoglobin. Due to observer's error, instrumental errors and cost effectiveness of Sahli's method we need to bridge the results variability and accuracy. Also it is not sufficiently accurate because it shows wide scatter in inter observers and intra observer results distribution(Balasubramaniam and Malthi 1992, Mills and Meadowst 1998). The reliability of the method needs to be confirmed as unreliability widens the distribution of hemoglobin values and results in biases in estimates of prevalence of anemia and response to intervention for it (Barduagni et al. 2003).

Sahli's method:- The hemoglobin tube (STD 14.5gm = 100% concentration) was filled with N/10 hydrochloric acid (HCl) up to 2 gm marking. This graduated tube was placed in Sahli's Hemoglobinometer (Comparator with Brown glass). Blood sample obtained from capillary or venous blood was drawn in Sahli's pipette up to 20 μ l mark and added in hemoglobin tube containing N/10 HCl. The blood and acid are mixed with glass stirrer and allowed to stand for 5minutes for acid hematine formation. Drop by drop distilled water was added to dilute the acid hematine compound color till it matches with the standard color plates of the comparator. Results were read as gm/dl present on the hemoglobin tube (Barduagni et al. 2003). Laboratory diagnosis in men is based on Hb<13-14g/dl, while in women it must be less than 12-13 g/dl (Janz et al. 2013, Smith 2010). (Annex V).

Pre-pregnancy counseling, dietary advice and therapy are very important for ensuring best pregnancy outcomes. It is recommended that full blood count should be checked at the booking

visit in pregnancy and repeated at 28 weeks to screen for anemia. In high risk mothers and multiple pregnancies, an additional hemoglobin check should be performed near term. Dietary advice should be given to all mothers to improve intake and absorption of iron from food. Rich sources of iron include hem iron (in meat, poultry, fish, and egg yolk), dry fruits, dark green leafy vegetables (spinach, beans, legumes, lentils) and iron fortified cereals. Using cast iron utensils for cooking and taking iron with vitamin C (orange juice) can improve its intake and absorption (Brooker 2000, Hughes 1991).

Increased intake of iron treatment of underlying conditions like de-worming (anti-helminthes therapy) are important preventive measures. Pregnant women need iron to cover their basic losses, increased RBC mass and demand from fetoplacental unit. Correction of iron deficiency in pregnancy involves appropriate diet and oral iron supplementation. Daily oral iron (60 mg) and folic acid (4 mg) should be commenced as soon as possible together with behavior changing communications when a woman becomes pregnant, and continued up to 6 months' postpartum. The dose of iron could be reduced to 30 mg in women who have no IDA. The aim is to achieve hemoglobin of at least 10g/dl at term. The choice of iron preparation is based on patient tolerance to a large extent. It is recommended to take iron with orange juice to enhance its absorption (Brooker 2000).

4. Materials and Methods

4.1 Study area

The study area was Kelela town which is situated at 10⁰28'N latitude and 38⁰48'E longitude in northeast Ethiopia (Amhara online.com). Kelela is at a distance of 561km from Addis Ababa. Specifically the study was based at Kelela health center (KHC). Kelela is one of the woredas in the Amhara region, part of south wollo zone. Kelela bordered in the south by the Semien Shewa Zone, in the southwest by the Woleqa river which separates it from the Oromia region, in the west by Wegde, in the northeast by Legehida, in the east by Jama. Towns in Kelela include kelela and Liguama (CSA 2014). Based on the 2014 population projection of Ethiopia for all regions at woreda level conducted by central statistical agency of Ethiopia (CSA), this woreda had a total population of 155,439 of whom 77,015 were men and 78,424 women; 11,313 or 7.27% were urban inhabitants. Kelela *woreda* and the study area are indicated in figure 1.

4.2 Study design and population

The study was a cross-sectional survey to assess the prevalence of anemia and its risk factors among pregnant women attending antenatal care (ANC) at KHC. So the source population consisted of all pregnant women in Kelela woreda visiting the ANC at KHC between January and May 2016. Pregnant women who were taking iron supplement during the survey, seriously ill and those who were unwilling to participate were excluded.

4.3 Data collection procedure

4.3.1 Questionnaire

A structured questionnaire (Appendix III) was used to obtain socio-demographic information, present and past history in pregnant women and clinical feature of anemia. The questionnaire was developed in Amharic and then translated into English. KHC nurses obtained training on data collection procedure to attain standardization and maximize interviewer reliability. The data collectors were regularly supervised by the principal investigator for proper data collection.

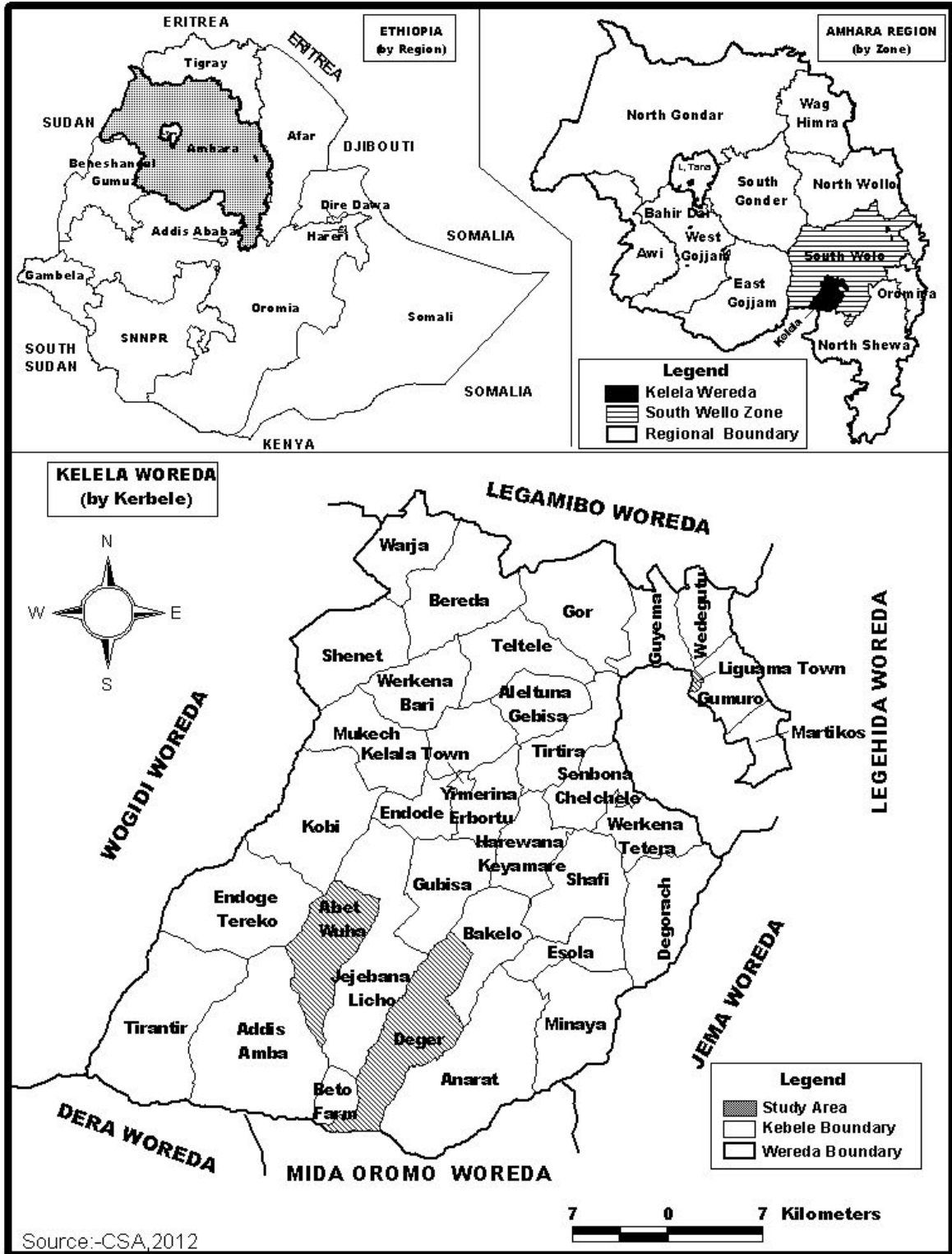


Figure 1 Map of Kelela woreda showing the study site (CSA 2007)

4.3.2 Hemoglobin determination

Venous blood samples were collected by practicing laboratory technicians for Hb measurement using the Sahli-Helling method. The Hb tube (STD 14.5gm = 100% concentration) was filled with N/10 hydrochloric acid (HCL) up to 2 gm marking. This graduated tube was placed in Sahli's Hemoglobinometer (Comparator with Brown glass). Blood sample obtained from capillary or venous blood was drawn in Sahli's pipette up to 20µl mark and added in hemoglobin tube containing N/10 HCl. The blood and acid are mixed with glass stirrer and allowed to stand for 5minutes for acid hematin formation. Drop by drop distilled water was added to dilute the acid hematin compound color till it matches with the standard color plates of the comparator. Results were read as g/dl present on the hemoglobin tube (Barduagni et al. 2003). The severity of anemia was noted based on the Hb value according to the WHO definition as mild (10-10.9g/dl), moderate (7-9.9g/dl) and severe (<7g/dl) anemia (WHO 2008)

4. 4 Data analysis

Data was entered and statistical analysis performed using SPSS version 16 software (IBM). Univariate and multivariate logistic regression models were used to test the association between dependent (anemia status) and independent variables (socio-demographic, clinical and others), and statistical significance was considered at $p < 0.05$.

4.5 Ethical considerations

Administration of the health center approved the study. Support letter from the Department of Zoological Sciences, Addis Ababa University was obtained. The consent of the study participants was ensured and confidentiality maintained. Anemic women were referred to the attending health professionals for treatment and follow-up.

4.6 Data quality control

To assure the quality of the data the questionnaire was first edited by colleagues of the school to avoid any ambiguity on the questions. Two nurses were trained to administer the questionnaire and collect data to reduce possible observer bias. Experienced laboratory technicians, standardized procedure and instruments like microscope, test tube, hemoglobinometer (comparator) and also hydrochloric acid, distilled water, and Isopropyl alcohol as a reagent were used to collect data from laboratory results (Appendix V). Data accuracy was assured by every day on-site assure.

5. Results

5.1 Study population

Totally 243 pregnant women having mean age 31.5 years (range: 19-42) were enrolled. The majority 173(71.2%) were 19-34 years old. The rest 70(28.8%) were greater than 35. More than half 142 (58.4%) of the women were from rural and 101(41.6%) urban. Hundred fifty one (62.1%) women were illiterate, 63(25.9%) able to read/write and 29(12%) in grade 1-6. Eighty-eight (36.2%) were housewives and 87(35.8%) farmers. Over 90% were married. The mean monthly income of the women was 338.28 Ethiopia Birr (range 70-1000). A total of the attendants had no known case of disease on admission to the ANC clinic (infectious or otherwise). Concerning gravidity, 38(15.6%) women were primigravidae, and the rest 205(84.4) multigravidae. But 141 had history of blood loss in their previous pregnancy. Sixty eight (28%), 87(35.8%), 88(36.2%) were in their first, second and third trimester of pregnancy. Only 12(38.7%) of the respondents indicated that they used to eat animal food (milk and meat) daily and 82(74.5%) of them used to eat animal food once a month. 41(53.2%) and 43(78.1%) of the respondents indicated that they used to eat green leafy vegetables once a week and once a month respectively.

5.2 Anemia prevalence and risk factors

The overall prevalence of anemia was 58.8%. In terms of severity, moderate anemia was 85(59.2%), mild anemia was 58(40.8%), and there was no severe case. The proportion of anemic women (n=101) in Kelela town 33(32.6%) was significantly lower than ($p < 0.0001$) that in the surrounding rural residents, n=142, 110(77.4%). Prevalence of anemia among pregnant women with birth interval less than or equal to two years was 76.5% which is higher than prevalence of anemia among pregnant women with birth interval > 2 years (27.5%) (n=205). The prevalence of anemia among pregnant women who did not attend the current ANC regularly was much higher 153(63%) than women who did attend current ANC regularly 90(37%). Pregnant women who have had blood loss during previous pregnancy showed significantly higher anemia prevalence compared to those who did not 96(68%) and 21(32.8%) respectively. Place of delivery also showed difference in prevalence of anemia 104(67.9%) in pregnant women who delivered at home and 23(44.3%) in women who delivered at health institute. Moreover, anemia was significantly higher among the multigravidae (AOR 0.212, 95% CI 0.048-0.941, $p = 0.041$) and

those in their third trimester of pregnancy (AOR 6.582, 95% CI 1.851-23.401, p 0.004).

In summary; all socio-demographic or clinical variables, except marital status and occupation, had significant association with anemia in the Univariate analysis (table 1). Similarly all clinical and dietary habit variables showed statistically significant association with anemia. Frequency of eating animal food and green leafy vegetables after meal significantly reduced the risk of anemia among the women. Birth interval, residence, gravidity and trimester also showed statistically significant association with anemia in the multivariate logistic regression analysis (table 2).

Table 1 Univariate logistic regression analysis of socio-demographic, clinical and dietary variables in pregnant women attending ANC follow-up at KHC, January-May 2016 (N=243)

Variables	Alternatives	n	Anemic, n (%)	COR	95% CI	p-value
Age(year)	19-34	173	111(64.2)	3.356	1.1295-9.972	0.003*
	≥35	70	32(45.7)	1		
Education	illiterate	151	107(70.8)	21.076	6.065-73.236	0.000*
	read/write	63	33(52.3)	9.533	2.616-34.748	0.001*
Income (ETB) monthly	1-6	29	3(10.3)	1		
	<250	126	89(70.6)	7.872	3.621-17.113	0.000*
	250-500	70	43(61.4)	5.212	2.275-11.943	0.001*
Trimester	>500	47	11(23.4)	1		
	first	68	22(32.3)	1		
	second	87	51(58.6)	2.962	1.526-5.751	0.001*
Residence	third	88	70(79.5)	8.131	3.936-16.798	0.000*
	urban	101	33(32.6)	1		
Blood loss	rural	142	110(77.4)	3.437	0.080-0.250	0.000*
	yes	141	96(68)	2.496	1.475-4.226	0.001*
ANC attendance	no	64	21(32.8)	1		
	yes	90	45(50)	1		
Occupation	farmer	153	98(64)	3.228	1.878-5.547	0.000*
	housewife	87	51(58.6)	2.962	1.526-5.75	0.230
	merchant	88	70(79.5)	8.131	3.936-16.798	0.530
Marital status	married	68	22(32.3)	1		
	single	201	131(65.1)	1.941	0.782-4.819	0.153
Gravidity	single	42	12(28.5)	1		
	primigravidae	38	21(55.2)	1		
Delivery site	multigravidae	205	141(69.1)	0.916	0.345-1.803	0.000*
	home	153	104(67.9)	3.228	1.878-5.547	0.000*
Birth interval(year)	health facility	52	23(44.2)	1		
	≤2	136	104(76.5)	8.444	4.689-15.207	0.000*
Frequency of eating animal food	>2	69	19(27.5)	1		
	daily	31	12(38.7)	1		
	every 2 days	42	21(50)	1.060	0.423-2.655	0.901
	every 2 weeks	60	28(46.6)	0.707	0.277-1.803	0.467
Frequency of eating green leafy vegetables	once a month	110	82(74.5)	0.226	0.091-0.563	0.001*
	daily	61	30(49.1)	1		
	every 2 days	50	29(58)	1.432	0.622-3.097	0.361
	every 2 weeks	77	41(53.2)	1.181	0.591-2.361	0.638
	once a month	55	43(78.1)	3.180	1.448-6.985	0.004*

ANC: antenatal care, CI: confidence interval, COR: crude odds ratio, ETB: Ethiopian Birr, n: people, %: percentage, * statistically significant, KHC: Kelela health center

Table 2 Multivariate logistic regression analysis of anemia by socio-demographic, clinical feature and dietary habit determinants in pregnant women attending antenatal care at KHC, January-May 2016 (N= 243)

Variables	Alternatives	N	Anemic, n (%)	COR	AOR	95 % CI	p-value
Age(year)	19-34	173	111(64.2)	2.588	3.099	0.925-10.330	0.066
	≥35	70	32(45.7)	1	1		
Education	Illiterate	151	107(70.8)	21.076	2.616	0.363-18.860	0.340
	read/write	63	33(52.9)	9.533	1.900	0.266-13.540	0.522
	1-6	29	3(10.3)	1	1		
Income(monthly ETB)	<250	126	89(70.6)	7.872	3.059	0.729-12.840	0.127
	250-500	70	43(61.4)	5.212	1.914	0.426-8.610	0.379
	>500	47	11(23.4)	1	1		
Blood loss	yes	141	96(68.0)	2.496	1.941	0.782-4.819	0.153
	no	64	21(32.8)	1	1		
Gravidity	primigravidae	38	21(55.2)	1	1		
	multigravidae	205	141(69.1)	0.916	0.212	0.048-0.941	0.041*
Delivery site	home	153	104(67.9)	8.440	0.719	0.264-1.957	0.518
	health facility	52	23(44.3)	1	1		
Birth interval	≤2 yrs	136	104(76.5)	8.440	2.235	8.427-8.677	0.000*
	>2 yrs	69	19(27.5)	1	1		
Trimester	first	68	22(32.3)	1	1		
	second	87	51(58.6)	2.962	3.704	1.096-12.520	0.035*
	third	88	70(79.5)	8.131	6.582	1.851-23.400	0.004*
ANC attendance	yes	90	45(50.0)	1	1		
	no	153	98(64.0)	3.228	1.367	0.536-3.486	0.531
Residence	urban	101	33(32.6)	1	1		
	rural	142	110(77.4)	3.437	5.182	1.943-13.820	0.001*
Frequency of eating animal food	daily	31	12(38.7)	1	1		
	every 2 days	42	21(20.0)	1.060	2.030	0.443-9.299	0.251
	every 2 week	60	28(46.6)	0.707	0.260	0.059-1.144	0.730
	once a month	110	82(74.5)	0.226	0.150	0.035-0.672	0.013*
Frequency of eating green leafy vegetables	Daily	61	30(49.1)	1	1		
	every 2 days	50	29(58.0)	1.432	2.361	0.544-10.240	0.251
	every 2 weeks	77	41(53.2)	1.181	1.265	0.333-4.805	0.730
	once a month	55	43(78.1)	3.180	4.700	1.192-18.520	0.027*

ANC: antenatal care, OR: adjusted odds ratio, CI: confidence interval, COR: crude odds ratio, n: no of people, KHC: Kelela health center, *statistically significant

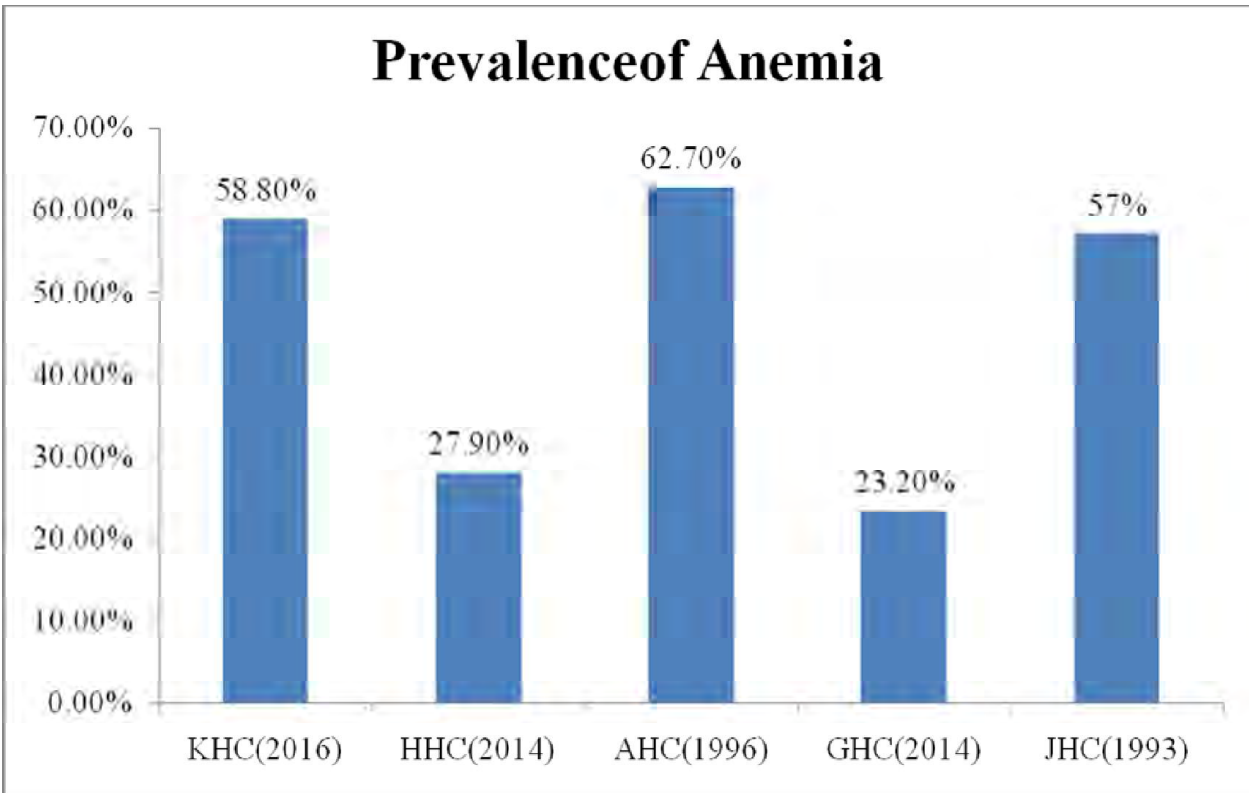


Figure 2 over all prevalence of anemia among pregnant women attending ANC at Kelela health center and other different parts of Ethiopia.

Key

- KHC Kelela health center
- HHC Harar health center
- AHC Asendabo health center
- GHC Gondar health center
- JHC Jimma health center

6. Discussion

The 58.8% prevalence of anemia obtained in this study was ‘medium’ and comparable with other local studies carried out in different parts of Ethiopia (Desalegn 1993, Getachew et al. 2012, Lelissa et al. 2015). Similar findings were also reported in eastern Sudan in sub-Saharan Africa (62.6%) (Prema et al. 1981). However, higher figures have been reported from China (70%) (Xing et al. 2009) and India (92.39%) (Kalaivani 2010).

However, the prevalence of anemia reported in pregnant women showed variability both locally within a country and between countries. In studies from southeast (Harar) and northeast Ethiopia much lower anemia prevalence was documented among pregnant women. This might be due to difference in the socio-demographic factors and lack of awareness about the consequences of anemia in this study. In this study, pregnant women on iron supplementation were not included, while these were included in studies done in Harar and Gondar. In other sub-Saharan African countries like Nigeria (Oboro et al. 2002) a similarly low prevalence of anemia was found (23.2%). In Thailand (Olubukola et al. 2011) the finding was much lower (14%).

Although the risk factors are globally established for anemia during pregnancy, the relative impact of each factor varies from setting to setting. Many studies reported different socioeconomic, demographic and clinical characteristics of pregnant woman that may affect the magnitude of anemia (Elashirya et al. 2014). Anemia was associated with aspects of lower socioeconomic class such as low level of education, rural residence, not working or manual worker, low reported income. Other clinical characteristics such as gravidity, trimester of pregnancy, and other factors are shown to be risk factors for anemia (Belachew and Legesse 2006).

The prevalence of anemia in the rural women was significantly higher than prevalence of anemia in urban women. Similar results obtained in a study conducted at Asendabo and Mettue, anemia among pregnant women was consistently higher in rural women compare to the urban counter parts (Gebremedin 2004, Yonas 1996). The prevalence of anemia among pregnant women from rural areas was likely related to lack of awareness of adequate supply of micro nutrients during

pregnancy, anti natal care and health education. Similar results were found in Ethiopia (Desalegn 1993) and elsewhere other studies (Zavaleta et al. 2000, WHO 2001).

Further, a study conducted on pregnant women in India showed a statistically significant association between residence and anemia which is consistent with the current study (Bechuram et al 2006). This finding indicates the need for strengthening of interventions in rural areas women to create awareness of antenatal care, balanced diet during pregnancy and family planning.

The accuracy of the Hb measurement used should be taken in to account. In this study, the Hb value of the pregnant woman was done by the old comparative Sahli-Helling method, which is subjected to biases in dilution and reading of results. Nevertheless, the result obtained in this study was consistent with the characteristics of the study population, and findings from other regional and international reports.

According to the World Health Organization classification, 58(40.2%) of anemic pregnant women had mild anemia (Hb 10-10.9 g/dl) and 85(60.8%) of the anemic women had moderate anemia, (Hb 7-9.9 g/dl) in this study. Other studies in Ethiopia, reported a mild anemia ranging from 23% to 81%, (Jufar and Zewde 2014, Olugimi 2014) and moderate anemia from 17.9% to 74.3%. The current finding is also comparable with studies in other countries. The study reported in this paper, however, differs in that it didn't identify any cases of severe anemia.

The present study and other studies in Ethiopia indicated that moderate anemia constitutes a significant portion of anemia in pregnant women attending ANC clinics (Kefiyalew et al. 2014). In fact, moderate anemia causes considerable consequences such as loss of energy and function for these women. Activities such as child care and household management as well as job performance are affected. They are more vulnerable to infections and improvement from infections may be prolonged. The anemia can also result in risks to the mother and newborn (Bukar et al. 2008, Prema et al. 1981). Therefore, increased health education on risk factors and interventions to prevent the prevalence and severity of anemia among pregnant women should be a priority for mothers attending ANC.

Pregnancy with a short birth interval leads to IDA as iron requirements are substantially higher than the average (WHO 1992). Short birth interval increases risk for uterine rupture. There was a significant association between birth interval and anemia (odds ratio (AOR) 2.235, 95% confidence interval (CI):8.427-8.677, $p=0.000$). This result was consistent with a study conducted in Jimma hospital (Belachew 2006). Pregnant women with short birth interval less than or equal to two years were two times likely to be anemic. Appropriate time after each pregnancy for recuperation and replenishment of nutrient stores and circulating levels is a minimum of three to five years (<http://www.infoforhealth.org/pr/113edsum.shtml>). This finding indicates the need for strengthening of interventions related to child spacing and awareness especially in rural women.

In this study it was identified that anemia was significantly higher in the multigravidae and third trimester of pregnancy. This is consistent with other studies conducted in sub Saharan Africa. This study also reported an association of gestational age with anemia and found that those in their third trimester were at significantly higher risk of anemia. This report is consistent with the work of others in Ethiopia and Nigeria (Olubukola et al. 2011, Niguse et al. 2013).

One of the major contributory factors in low-income countries is usual consumption of plant-based food containing insufficient iron (Van den Brock et. al 2000). Eating animal food and leafy green vegetables showed a statistically significant association with reduced risk of anemia in this study. The study also identified at women at increased risk of anemia for targeted intervention. For example, more focus must be given for multigravidae women than premigravidae and for women in the third trimester of pregnancy in ANC follow-up.

The major challenge in this study was low literacy level of the participants especially in the villages. More than 70% of the respondents could not read/write. Thus much time was spent in explanations and illustrations were made for better understanding and responses. The method used to measure hemoglobin level was Sahli Tube method which can result in personal bias during comparing with the standard due to the lack of well organized laboratory facilities in Kelela health center.

7. Conclusion and Recommendation

Anemia remains a significant public health concern in the study area with relatively more number of cases in rural kebeles than Kelela town. Established anemia risk factors were highly prevalent in the area. The prevalence of anemia was high among women in their third trimester, who were multigravidae, having lesser birth intervals and those who did not attend ANC regularly. Although the condition was moderate control interventions including health education should be scaled-up to achieve better. There is a need for early counseling and screening for anemia and its cause(s) to take prompt prophylactic or therapeutic intervention. Pregnant women should be reached out and encouraged to develop a tradition of regular/early ANC follow-up.

8. References

- Anemia during pregnancy. <http://americanpregnancy.org/pregnancy-concerns/anemia-during-pregnancy>. Retrieved August 2016.
- Ayene F, Abere Y, Timerga G 2014. Pregnancy anemia prevalence and associated factors among Women Attending ante natal care in Debre Berhan health institutions, Ethiopia. *J Women's Heal*; **3**:173.
- Balasubramaniam P, Malathi A 1992. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *J Postgrad Med*; **38**:8-9.
- Barduagni P, Ahmed A, Curtale F, Raafat M, Soliman L 2003. Performance of Sahli' sand Color scale method in diagnosing anemia among school children in low prevalence areas. *Trop Med Intl Heal*; **8**:615-18.
- Beaton G 2000. Iron needs during pregnancy: do we need to rethink our targets? *Amer J Clin Nutri*; **72**:265-71.
- Bechuram M, Vikal T, Ranjan G 2006. Risk factors of anemia during pregnancy among the Garo of meghalaya, India. *Human Eco Specl Issu*; **14**:27-32.
- Belachew T, Legesse Y 2006. Risk factors for anemia among pregnant women attending antenatal clinic at Jimma University, Hospital, southwest Ethiopia. *Ethiop Med J*; **44**:211-20.
- Booth M 1998. Associations among multiple geo-helminthes species infections in school children from Pemba Island. *Parasitol*; **116**:85-93.
- Bothwell H 2000. Iron requirements in pregnancy and strategies to meet them. *Amer J Clin Nutri*; **72**:257-64.
- Brooker S 2000. Epidemiology of single and multiple species of helminthes infections among school Children in Busia District, Kenya. *East Afr Med J*; **77**:157-61.
- Bukar M, Audu B, Yahaya U, Melah G 2008. Anemia in pregnancy at booking in Gombe, North-eastern Nigeria. *Gynaecol*; **28**:775-8.
- Causes of anemia [http:// www.marckmanuals.com](http://www.marckmanuals.com) Accessed on June 15, 2016.
- CSA 2007. Population and housing census of Ethiopia. Addis Ababa Ethiopia: population census commission central statistical agency.
- CSA 2014. Population projection of Ethiopia for all regions at wereda level from 2014-2017.

- Addis ababa. Ethiopia: population projection central statistical agency.
- De Benoist B, McLean E, Egli I, Cogswell M 2008. Worldwide prevalence of anemia 1993-2005: WHO global database on anemia, WHO, Geneva.
- Desalegn S 1993. Prevalence of anemia in pregnancy in Jimma town, southwestern Ethiopia. *Ethiop Med J*; **31**:251-8.
- Diagnosis of anemia. <http://www.wbdl.com> Accessed on April, 2016
- Elashirya A, El ghazali S, Habil I 2014. Prevalence and determinants of anemia in third trimester Pregnancy in fayoum governorate-Egypt. *Acta Medica Medite*; **30**:1045.
- Filagot Kefiyalew, Endalew Zemene, Yaregal Asres, Lealem Gedefaw 2014. Anemia among pregnant women in Southeast Ethiopia: prevalence, severity and associated risk Factors. *BMC Research Notes*; **7**:771.
- Gebremedin S 2004. Prevalence of anemia and its predisposing factors in pregnant mothers attending antenatal care in Mettu Carl hospital south western Ethiopia. *Ethiop Med J*; **31**:251-8.
- Getachew M, Yewhalaw D, Tafess K, Getachew Y, Zeynudin A, 2012. Anemia and associated risk factors among pregnant women in Gilgel Gibe dam area, Southwest Ethiopia. *Parasit Vectors*; **5**:296.
- Hughes A 1991. Anemia pregnancy. Maternal health and safe motherhood. Division of family Health, WHO.
- Iron deficiency Anemia Mayo clinic. March 4, 2011. Retrieved December, 2016.
- Iron deficiency Anemia. Medline plus, Medical encyclopedia, <http://www.marchofdiems.org> Retrieved February 2016.
- Iron deficiency anemia: assessment, prevention, and con-troll. A guide for programme managers. Geneva, World Health Organization, 2001.
- Janz T, Johnson R, Rubenstein S 2013. Anemia in the in the emergency department: evaluation and treatment. *Emerg Med Prc*; **15**:1-15.
- Johns L, Lewis M 1989. Primary health screening haemoglobinometry in a tropical community. *Bull WHO*; **67**:627-33.
- Jufar A, Zewde T 2014. Prevalence of anemia among pregnant women attending antenatal care at Tikur Anbessa specialized hospital, Addis Ababa Ethiopia. *Hematol Thromb Dis*; **2**:1.

- Kalaivani K 2010. Prevalence & consequences of anemia in pregnancy. *Indian J Med Res; (AMJ)*; **3**:276-80.
- Lelissa D, Yilma M, Shewalem W, Abraha A, Worku M, Ambachew H, Birhaneselassie M 2015. Prevalence of anemia among women receiving antenatal care at Boditii health center, Southern Ethiopia. *Clin Med Res*; **4**:79-86.
- Melku M, Addis Z, Alem M, Enawgaw B 2014. Prevalence and predictors of maternal anemia during pregnancy in Gondar, Northwest Ethiopia: An institutional based cross-sectional study, anemia. Hindawi Publishing Corporation; Article ID 108593.
- Mills A, Meadowst N 1998. Screening for anemia: evaluation of a hemoglobinometer. *Archives of Disease in childhood*; **64**:1468-71.
- Nasreen H, Ahmed M, Begum A, Afsana K 2010. Maternal, neonatal and child health Programmes in Bangladesh: review of good practices and lessons learned. Series 32 research monograph. Dhaka: BRAC Center.
- Niguse O, Mossie A, Gobena T 2013. Magnitude of anemia and associated risk factors among pregnant women attending antenatal care in shalla woreda, west Arsi zone, oromia region, Ethiopia. *Ethiop J Health Sci*; **23**:165-73.
- Oboro V, Tabowei T, Jemikalajah J 2002. Prevalence and risk factors for anemia in pregnancy in South Southern Nigeria. *J Obstet Gynaecol*; **22**:610-13.
- Olubukola A, Odunayo A, Adesina O 2011. Anemia in pregnancy at two levels of Health Care in Ibadan, southwest Nigeria. *Anna Afr Med*; **10**:272-77.
- Olujimi A, Bosun O, Aniekan M, Abasiattai B, Emem A, Basseyy R, James S, Ibanga G, Anyiekere M 2014. Prevalence of Anemia among Pregnant Women at Booking in the University of Uyo Teaching Hospital. Uyo, Nigeria. *Bio Med Res Int*; **10**:22-25
- Oppenheimer L, Sherriff E, Goodman J, Sheh D, Jems C (1986). The duration of lochia. *Br J Obstet Gyanecol*; **93**: 754-57.
- Poggi S 2013. Postpartum hemorrhage and abnormal puerperium. In: DeCherney H, Nathan L, Goodwin M, Laufer N, (Eds); *J Med*; **358**:888-99.
- Prema K, Kumari S, Ramalakshmi A (1981). Anemia and adverse obstetric out come. *Nutr Rep Int*; **981**:637-43.
- Reproductive Health. New findings on birth spacing: three to five years is the optimal interval,

- <http://www.infoforhealth.org/pr/113edsum.shtmle> server programs birth spacing optimal interval. Accessed on may 5, 2016.
- Rodak T, Bernadette F 2007. Hematology: clinical principles and applications (3rd Ed.). Philadelphia: *Saunders*; 220.
- Sewpersad N, Inkosi G 2010. Thalassemia, Albert Luthuli Central Hospital, and Sunil S, Chairperson: South African Thalassemia Association.
- Smith RE 2010. The clinical and economic burden of anemia. *The Amer J Mana care*; 59-66.
- Sukrat B, Suwathanapisate P, Siritawee S, Pongthong T, Phupongpankul K 2010. The prevalence of iron Deficiency anemia in pregnant women in Nakhonsawan, Thailand. *J Med Assoc Thai*; **93**:765-70.
- The seventh types of anemia.mhealth24.com Reviewed June, 2010. Retrieved on March 4, 2016.
- Van den Broek R, Rogerson J, Mhango G, Kambala B, White A, Molyneux M 2000. Anemia in pregnancy in southern Malawi: prevalence and risk factors. *BJOG: Intl J Obst Gynec*; **107**:445-51.
- Van Schenck H, Falkensson M, Lundberg B 1986. Evaluation of “HemoCue” a new device for determining hemoglobin. *Clin Chem*; **32**:526-29.
- Villanova A 1994. Reference and selected procedures for the quantitative determination of hemoglobin in blood: approved standards. 2nded. National committee for clinical laboratory standards. *J pharm*; **5**:30-34.
- Vos T, Flaxman D, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon J, et al. 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 Diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*; **380**:2163-96.
- WHO 1992. The prevalence of Anemia in women: A tabulation of available Information. World Health Organization, Geneva, Switzerland.
- WHO 2002. *The World Health Report 2002: Reducing risks, promoting healthy life*. World Health Organization, Geneva, Switzerland.
- WHO 2015. Micronutrient deficiencies: Prevention and control guidelines, World Health Organization, Geneva, Switzerland.
- WHO, CDC 2008. Benoist B, McLean E, Egli I, Cogswell M (Eds) Worldwide Prevalence of

Anemia 1993-2005. Geneva: World Health Organization.

Xing Y, Yan H, Dang S, Zhuoma B, Zhou X, Wang D 2009. Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study. *BMC Pub Heal*; **9**:336.

Yonas T 1996. Prevalence of anemia among ANC attendants of Asendabo teaching health center. Jimma, Ethiopia: MSc thesis. Jimma Institute of Health Sciences.

Zavaleta N, Caulfield L, Garcia T 2000. Changes in iron status during pregnancy in Peruvian women receiving prenatal iron and folic acid supplements with or without zinc. *J Amer Nutri*; **71**:956-61

9. Annexes

Annex 1 Consent form

Code number_____

This is an agreement request to assess prevalence of anemia in pregnant women. With this request those pregnant women who are attending antenatal follow up at kelela health center and willing to participate in the study will be invited to assess determinants of anemia. Based on the laboratory investigation anemic and pregnant women will be treated free of any cost.

Objective of the study

Questionnaires will be requested on determinants of anemia to assess anemia. On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose. You have every right to accept or refuse participation of this study.

You will be requested to give small amount of blood. Blood will be collected from the tip of your finger using sterile lancet. There will be some pain during pricking of your finger but not harmful to your health. If you are agree to give samples you will be requested to answer for questionnaire.

Are you willing to participate with the study by giving blood sample and answer to the request?

A Yes B No

THANK YOU

Name of health institution _____

Patient name _____ signature _____ date _____

Name of data collection _____ signature _____ date _____

Annex 2 Amharic version of the consent form

የወል ስምምነት ቅፅ

መለያ ኮድ-----

በአዲስ አበባ ዩኒቨርሲቲ የሳይንስ ፋካልቲ የዙዮሎጂካል ትምህርት ክፍል፡

በመጠይቁም በከለላ ጤና አጠባበቅ ጣቢያ አገልግሎት ከሚያገኙ ነፍሰጡር እናቶች ውስጥ በጥናቱ መካፈል ፈቃደኛ የሆኑ እንድሁም መመዘኛውን በሚያማሉት ላይ የደም ማነስ መንስኤዎችና አጋላጭ ሁኔታዎች ለደም ማነስ ስርጭት ያላቸው እገዛ ምን እንደሚምስል ለማጥናት የተዘጋጀ ወል ነው። የደም ናሙና ምርመራ በማድረግ በሚገኘው ወጤት መሰረት የደም ማነስ ችግር ካለብዎት ከጤና ጣቢያዎ ላለሙያዎች ጋር በመተባበር ተገቢውን የህክምና አገልግሎት ያገኛሉ።

የጥናቱ አላማ

ለደም ማነስ መንስኤ እና አጋላጭ በሆኑ ተፅዕኖች ዙሪያ ቃለ መጠይቅ ይቀርብሎቻል በመጠይቁም ላይ ስምዎትን ወይም የርሰዎን ማንነት የሚገልፅ ማንኛውም ነገር አይጠቀስም ወይም አይያያዝም። የሚሰጡትም መረጃ ሆነ ናሙና ከዚህ ጥናት ወጭ ሆነ ከርሰዎ ጋር ለተገናኘ ለሌላ ጥቅም በፍፁም አይወልድም።

በዚህ ጥናት የደም ናሙና ለመስጠት እንድሁም ለሚቀርብሎት ቃለ-መጠይቅ ምላሽ በመስጠት ሙሉ ተሳታፊ ለመሆን ፈቃደኛ ነዎት?

አዎ ከሆነ መልሰዎ

ስለ ትብብርዎ እናመሰግናለን

የጤና ተቋሙ ስም-----

የጥናቱ ተካፋይ ስም----- ፊርማ----- ቀን-----

የመረጃ ሰብሳቢዉ ስም----- ፊርማ----- ቀን-----

Annex 3 Questionnaire

Prevalence of anemia among pregnant women attending ante natal care at Kelela health Center

Part-1-socio demographic and economic characteristics of respondents

Code No _____ age _____

Address: region _____ zone _____ woreda _____ kebele _____

1. How old are you?
2. Occupation
A. farmer B. house wife C. merchant
3. Educational status
A. Illiterate B. read and writes C. 1-6
4. What is your monthly income (in average) in birr?
A. ≤ 250 B. 250-500 C. > 500
5. Where do you live? A. urban B. rural
6. What is your marital status? A. married B. single

Part 2 Knowledge and associated factors of anemia

7. What is the duration of time you waited between pregnancies?
A ≤ 2 years B. > 2 years
8. Did you follow ANC during current pregnancy? A. yes B. No
9. Do you eat meat and animal products? A yes B No
10. If you eat meat and animal products how many times?
A daily B every 2 days C. every weeks D once a month
11. Do you eat green leafy vegetables? How many times?
A daily B every 2 days C every weeks D once a month
12. What is your gravidity? A primigravidae B multigravidae
13. Where do you deliver your babies? A health center B home
14. Was there any blood loose in your previous delivery? A yes B No
15. Was there any blood losses in your current pregnancy? A Yes B No
16. What is your gestational age? A first trimesters B second trimesters C third trimesters

Annex 4 Amharic version of questionnaire

በአዲስ አበባ ዩኒቨርሲቲ የሳይንስ ፋካልቲ የዙዮሎጂካል ትምህርት ክፍል ሙሉ ፈቃደኛ በሆኑ ነፍሰጡር እናቶች ላይ የሚከሰት ደም ማነስ መንስኤዎች እና አጋላጭ ሁኔታዎችን በተመለከተ ለሚደረግ ጥናት የተዘጋጀ መጠይቅ፡

በዚህ ጥናት ለመካፈል የደም ናሙና ለመስጠት እንድሁም ለሚቀርብሎት ቃለ-መጠይቅ ምላሽ በመስጠት ሙሉ ተሳታፊ ለመሆን ፈቃደኛ ነዎት?

አዎን----- አይደለም-----

የጤና ተቋም ስም-----

የጥናቱ ተካፋይ መለያ ኮድ-----

ስነ-ማህበራዊ እና ህዝባዊ መጠይቅ

1. እድሜዎት ስንት ነው?

2. ስራዎት ምንድን ነው? ሀ. ገበሬ ለ. የቤት እመቤት መ. ነጋደ

3. የትምህርት ደረጃዎ ስንት ነው?

ሀ. ማንበብና መጻፍ አልችልም ለ. ማንበብና መጻፍ እችላለሁ መ. ከ1-6ኛ ክፍል

4. የወር ገቢዎ ስንት ነው?

5. የት ነው የሚኖሩት? ሀ. ገጠር ለ. ከተማ

6. የጋብቻ ሁኔታ? ሀ. ያገባች ለ. ያላገባች

7. ልጆችን በምን ያህል ጊዜ አራርቀው ይወልዳሉ? ሀ. ከሁለት አመት በታች ለ. ሁለት አመት እና ከዚያ በላይ

8. የቅድመ-ወሊድ ክትትል ያደርጉ ነበር? ሀ. አዎ ለ. አላደረኩም

9. ስጋ እና የእንስሳት ውጤቶችን ይመገባሉ? ሀ. አዎ ለ. አልመገብንም

10. ስጋ እና የእንስሳት ውጤቶችን የሚመገቡ ከሆነ ለምን ያህል ጊዜ?

ሀ. በየቀኑ ለ. ከሁለት ቀን አንደ መ. በሳምንት አንደ ሠ. በወር አንደ

11. አረንጓዴ ቅጠላ ቅጠሎችን ይመገባሉ? ሀ. አዎ ለ. አይደለም

12. አረንጓዴ ቅጠላ ቅጠሎችን የሚመገቡ ከሆነ ለምን ያህል ጊዜ?

ሀ. በየቀኑ ለ. ከሁለት ቀን አንደ መ. በሳምንት አንደ ሠ. በወር አንደ

13. ከዚህ በፊት የወለዱዎቻቸው ህፃናት የት ነው የተወለዱት? ሀ. ጤና ተቋም ለ. ቤት

14. በወሊድ ጊዜ የደም መፍሰስ ችግር አጋጥሞዎት ያወቃል? ሀ. አዎ ለ. አያወቅም

15. ለስንት ጊዜ አርግዘዋል?

16. ካረገዙ ስንተኛ ወርዎ ነው?

Annex 5 procedure of hemoglobin estimation by sahli's-healing method (sahli's hemoglobinometer)

Materials used

1. sahli's hemoglobinometer (comparator)
2. comparison tube
3. 0.02ml pipette (hemoglobin pipette with rubber tubing and mouthpiece)
4. 2 Pasteur pipettes (one for HCl and one for distilled water)
5. Glass rod to stir (stirrer)
6. Hydrochloric acid
7. Distilled water

Procedures

1. Add hydrochloric acid (1: 10 diluted) to the hemometer tube (comparison tube) up to lowest graduation (0.02 gram)
2. Sterilize the fingertip with isopropyl alcohol surgical spirit and allow it to dry
3. Using sterile lancet prick the finger tip
4. Wipe away first few drops of blood
5. Suck blood in to the hemoglobin pipette (capillary pipette) up to 20cu.mm (avoid air bubbles come in to a tube)
6. Wipe the outside tip of pipette clean with tissue
7. Immediately transfer the blood to the comparison tube
8. Suck blood back in to the pipette several times and blow out again in to the tube (to mix blood with HCl)
9. Place the hemometer tube in the stand and allow for 10 minutes (during this period HCl lysis red cell and released hemoglobin on reacting with forms a dark brown colored acid hematine)
10. Now using a pasture pipette to add a few drops of distilled water and stir the contents with a glass rod.
11. Continue to add water drop by drop and stir the contents each time until the solution is just darker than the standard.
12. Carefully add one or two drops of water till the color exactly matches with that of the standard and note the reading.

13. While taking the reading hold the hemoglobinometer against good daylight at arm length.
14. The comparison tube represents 100 percent with reference to a standard which is 14.8g HB/100 ml of blood.

Annex 6 pictures of sample collection and facilities of the laboratory in KHC



Annex 7 Ethical clearance

ቀን 27/12/2007

ቁጥር:- SF/ZS/1491/07/2015

ለሚመለከተው ሁሉ

ጉዳዩ:- ትብብር ስለመጠይቅ

ተማሪ በአዲስ አበባ ዩኒቨርሲቲ የ zoological science ት/ክፍል የክረምት መርሀ ግብር የባዮሎጂ MSc ተማሪ ሲሆን በ2008 ዓ.ም. የMSc thesis ምርምር የሚያካሂዱ በመሆናቸው በመ/ቤትዎ በኩል አስፈላጊው ትብብር ይደረግላቸዋል ዘንድ በትህትና እንጠይቃለን።

ከሰላምታ ጋር

የዘሎጂካል ሳይንስ ትምህርት ክፍል

10. Declaration

I, the undersigned, declare that this Thesis is my original work and all source materials used are duly acknowledged.

Name Aragaw Mohammed

Signature _____

Date _____

11. Statement of the supervisor(s)

This Thesis has been approved for submission to the Department of Zoological Sciences for public defense.

Name Hassen Mamo (PhD)

Signature _____

Date _____