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(Physiology)

Assessment of Association between Helicobacter Pylori Infection and Occurrence of Anemia among Pregnant Women attending Antenatal care in Kulito Health Center, Halaba Special Woreda, SNNPR, Ethiopia, 2018

By:

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Abstract

Background: *Anemia in pregnancy is defined as a hemoglobin (Hb) concentration of less than 11gram (gm)/deciliter (dl) in venous blood. Globally it affects 1.62 billion people. According to World Health Organization (WHO) estimates, up to 56% of all women living in developing countries are anemic. In Ethiopia, the prevalence of anemia is 17% in the reproductive age group of women and 22% among pregnant women according to Ethiopian demographic and health survey report in 2016. In developing countries anemia is a major cause of maternal and child morbidity and mortality. Globally anemia contributes for 20% of all maternal deaths. Determining the magnitude and associated factors of anemia among pregnant women in a specific setting would help scale-up preventive and therapeutic measures of anemia. However, this information especially about the effects of HP infection on anemia is limited generally in Ethiopia and particularly in the study area.*

Objective: *The aim of this study was to determine the association between anemia and HP infection among pregnant women attending antenatal care follow up in Kulito Health Center, Halaba Special Woreda, SNNPR, Ethiopia, 2018.*

Material and Methods*: An institution based cross-sectional study was conducted on a total of 236 participants. An interviewer administered pre-tested structured questionnaire was used to collect data. Measure mid upper arm circumference (MUAC) of the participants was measured. Venous blood and stool samples were collected and analyzed for determination of Hb concentration, serum HP antibody status and intestinal parasites infestation of the participants. SPSS version 21.0 (SPSS Inc., Chicago, USA) was used for data analysis. Descriptive statistics and logistic regression were carried out to compute frequencies, means, proportion and relevant associations. From multivariate logistic regression, independent variables having a p-value <0.05 at 95% CI were considered as statistically significant.*

Results: Result: *Among 236 pregnant women, the prevalence of anemia was found to be 65(27.5%) with 36(15.2%) of mild, 29(12.3%) of moderate and no severe cases of anemia. The overall prevalence of HP infection among study participants was found to be 129(54.7%) (95% CI: 47.9-61.4). Pregnant women with HP infection were 3 times (AOR=3.064, 95% CI: 1.336-7.027) higher to develop anemia as compared to those without HP infection. Study participants who have low inter-pregnancy gap (IPG < 2 years) were 2.8 times (AOR=2.863, 95%CI: 1.245-6.582) more likely to suffer from anemia when compared to having IPG ≥ 2 years. Pregnant women who were in the third trimester were 6.5 times (AOR=6.457; 95% CI: 1.276-32.729) higher to develop anemia as compared to those in the first trimester. Study participants with MUAC level <21cm was 2.6 times (AOR=2.595, 95%CI: 1.044-6.450) more likely to develop anemia as compared to participants with MUAC level ≥ 21cm.*

Conclusion: *The present study revealed that the overall prevalence of anemia and HP infection was 27.5% and 54.7% respectively among pregnant women attending antenatal care follow up in Kulito health center. HP infection, IPG, gestational age being in third trimester and MUAC level were the predictors of anemia.*

Key words: *Anemia, HP infection, Pregnancy, HP serum antibody test****,*** *Kulito Health Center.*

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Abbreviations and acronyms

ANC…….…Antenatal Care

AOR…… Adjusted Odds Ratio

CI…….Confidence Interval

COR…… Crude Odds Ratio

Dl…….….Deciliter

Fe^{2+}…….……..Iron Ion

Gm…….…. Gram

Hb…...……..….. Hemoglobin

HC…..…….Health Center

HIV….…….……Human Immune Virus

HP…… Helicobacter Pylori

ID…….. Iron Deficiency

IDA…….Iron Deficiency Anemia

IgG……..Immunoglobulin G

IP…….....Intestinal Parasite

IPG……..Inter Pregnancy Gap

LBW…...…….. .Low Birth Weight

MALT…… Mucosal Associated Lymphoid Tissue

MUAC……Mid Upper Arm Circumference

RBC…… Red Blood Cells

SNNPR……South Nation Nationalities and People Regional State

SPSS……Statistical Package for Social Science

WHO……World Health Organization

CHAPTER 1 Introduction

1.1 Back ground

Anemia is a decrease in the concentration of circulating red blood cells or in the haemoglobin (Hb) concentration and a concomitant impaired capacity to transport oxygen(1). Anemia in pregnancy is defined as a Hb concentration of less than 11gm/dl in venous blood(2). Hb is oxygen-carrying pigment of the red blood cells (RBCs). Each RBC contains about 280 million Hb molecules. One Hb molecule consists of a protein called globin. It has four poly peptide chains (two alpha and two beta chains); and a ring like non protein pigment called a heme which is bound to each of the four chains. At the center of each heme ring is an iron ion (Fe^{2+}) that can combine reversibly with one oxygen molecule, allowing each Hb molecule to bind four oxygen molecules(1). Each oxygen molecule picked up from the lungs is bound to an iron ion. As blood flows through tissue capillaries, the iron–oxygen reaction reverses. Hb releases oxygen, which diffuses first into the interstitial fluid and then into cells(3).

The normal range of adult non pregnant women Hb concentration is ≥12gm/dl(4). World health organization (WHO) has accepted up to 11gm/dl as the minimum normal Hb level in pregnant women. Therefore, any Hb level below 11gm/dl in pregnant women should be considered as anemia(5). Anemia is one of the most common nutritional deficiency diseases observed globally and affects more than a quarter of the world's population of which, under five children and pregnant women are highly vulnerable groups(6). Half of all the cases of anemia can be attributed to iron deficiency (ID) and WHO has reported that iron deficiency anemia (IDA) is the most prevalent nutritional problem, which is affecting more than a billion people(7).

In pregnancy, several physiological changes occur that lead to decrease in the level of Hb. Among these changes one is an increase in plasma volume by 50% while the red cell mass increases by only 33%; and consequently, there is a fall in Hb concentration with reduction in both the Hb and red cell count because of hemodilution(8).

In developing countries, the cause of anemia during pregnancy is multi factorial and includes nutritional deficiencies of iron, folate, and vitamin B12 and also parasitic diseases, such as malaria and hookworm(9). Another factor that is believed to contribute for the occurrence of anemia in pregnancy is a defect in gastric absorption and utilization of dietary or supplemental iron due to Helicobacter Pylori (HP) infection(10).

HP is a gram-negative microaerophilic bacterium that infects the epithelial lining of the stomach. It is etiologically associated with chronic active gastritis, peptic ulcer disease, gastric cancer and mucosal associated lymphoid tissue(MALT) lymphoma(11). Nearly 50% of the world's population is estimated to be infected with HP, but the prevalence is high in developing countries than developed ones, although many infected individuals are asymptomatic(12). It has been reported that high prevalence of HP is observed among pregnant women, which is associated with increased risk of anemia(13). It is hypothesized that HP-associated with anemia is caused by both compromised absorption of bio-available iron in the context of hypochlorhydria, and the competing iron demands of HP and the host(14). Bleeding through peptic ulcers and other gastric lesions due to bacterial irritation of gastric mucosal also contribute for the occurrence of anemia(15).

In developing countries, the risk factors of anemia during pregnancy are multi-factorial. This includes nutritional deficiencies, parasitic infection and sociodemographic and economic status of the mothers(9). Accordingly, age, place of residence, marital status, employment status, family size, educational background and wealth status are determinants of anemia(16).

Pregnancy is considered to be a delightful experience for the expectant mother. Evidences manifested that adequate intake of nutrition is a key component for individual's health and well-being, particularly during pregnancy. It is well documented that inadequate maternal nutrition results in increased risks of Intra Uterine Growth Restriction(IUGR), low birth weight (LBW), preterm birth, prenatal and infant mortality and morbidity(17).

During pregnancy, the pregnant mother undergoes significant physiological changes in order to nurture and accommodate the developing fetus. Gravidity, gestational age and inter pregnancy gap were important variables(18).

1.2 Statement of the problem

Anemia is a global public health problem affecting both developing and developed countries. Globally, it affects 1.62 billion people, among which 56 million are pregnant women(2,9). According to WHO estimates, up to 56% of all women living in developing countries are anemic(6). IDA among women in developing countries is 40% to 88%(7). In Ethiopia, the prevalence of anemia is 17% in the reproductive age group of women and 22% among pregnant women according to Ethiopian demographic and health survey report in 2016(19). But the prevalence of anemia nearby the present study area in Bodity, SNNPR in 2012 among pregnant women was 61.6%(20).

Anemia during pregnancy is a major cause of morbidity and mortality of pregnant women in developing countries and has both maternal and fetal consequences(2). Globally anemia has a major consequences in human health, social and economic development and contributes for 20% of all maternal deaths(12). Apart from maternal related complications, anemia in pregnancy may also lead to premature births, low birth weight, fetal growth impairment and infant deaths(21). Half of all the cases of anemia can be attributed to ID(22). WHO has reported that IDA is the most prevalent nutritional problem, which is affecting more than one billion people(23). The severity of the problem is more among women of reproductive age, especially in pregnant women(2).

In sub-Saharan Africa, ID is the most common causes of anemia in pregnant women and it affects the development of a country by decreasing the cognitive development of children and the productivity of adults(24). Despite routine iron supplementation and promotion of diet modification, anemia remains widely prevalent in pregnant mother(25). Many studies reported that HP infection, IP infestation, low IPG and nutritional status might play a potential role in the occurrence of anemia in pregnant women(26,27).

Hp infection is the most common chronic bacterial infection in humans worldwide(28). The global prevalence of HP infection is 20-50% in developed countries and 50-80% in developing one(29). Regionally the prevalence is; 7-30% in North America, 30-90% in South America, 1.2-70% in Europe, 13-90% in Asia, 50-94% in the Middle East, 15-20% in Australia, and 48-95% in Africa(30). A high prevalence of HP has been observed among

pregnant women. The study conducted in Turkey and Egypt showed the prevalence of HP in pregnant women was 44.8% and 88%, respectively(31). Cross-sectional study conducted in different part of Ethiopia among the general population showed that the prevalence of HP was; 83.3% in Hawassa, 50.7% in Jinka, 52.4% in Butajera and 71.0 % in Jigjiga(11,14,32,33). A comparative cross-sectional study done in Addis Ababa reported that the prevalence of HP was higher among pregnant women than non pregnant women 33.8 % versus 21.9 % respectively(13).

Low Hb concentrations indicative of moderate or severe anemia during pregnancy have been associated with an increased risk of premature delivery, maternal and child mortality, and infectious diseases(16). IDA may affect the growth and development of fetus in the uterus and after birth in the long term(15). Daily oral iron and folic acid supplementation is recommended as part of the antenatal care to reduce the risk of anemia and its complications(23). HP infection has been indicated as a cause of failure with oral iron treatment(10). Several ID mechanisms have been hypothesized in HP infection, some of which are decreased mucosal iron absorption capacity due to decrease gastric pH, reduction of stomach vitamin C levels, bacterium-host competition for dietary iron supply and lactoferrin mediated iron sequestration by gastric HP(25). In pregnant women, HP infection has been found to be associated with IDA(15).

A Case-control study showed that high prevalence of HP infection was seen in pregnant women suffering from IDA and eradication of the infection by triple drug therapy during third trimester enhanced the response to oral iron folic acid supplementation(25). Determination of the associated factors of anemia among pregnant women helps to monitor health of the pregnant women, contributing to reduction in maternal and child morbidity and mortality(21).

To the best knowledge of the investigator, there is a limited study conducted generally in Ethiopia and specifically in Halaba Special Woreda, about the association of anemia and HP infection among pregnant women.

1.3 Significance of the study

Anemia is the major cause for maternal morbidity and mortality. HP infections and other related factors such as obstetric history and nutritional status contribute for the occurrence of anemia among pregnant women. So prevention and treating of anemia and associated factors significantly reduce maternal and child morbidity and mortality. Anemia contributes for 40% of maternal death in developing country like Ethiopia. Anemia also contributes for premature births, low birth weight, fetal impairment and infant deaths. Apart from maternity-related complications, anemia has major consequences on human health and social and economic development. It adversely affects physical and cognitive development in children and may increase risk of psychiatric disorders among children and adolescents.

WHO and Federal Ministry Health of Ethiopia recommended iron supplement for all pregnant women. However, still there is the burden of anemia among pregnant women. So there may be another risk factor that contributes for the occurrence of anemia especially in pregnancy women. HP infection may contribute for the occurrence of anemia especially among pregnant women by decreasing mucosal iron absorption capacity due to decrease gastric pH, reduction of stomach vitamin C levels, bacterium-host competition for dietary iron supply and lactoferrin mediated iron sequestration by gastric HP.

CHAPTER 2 Literature review

2.1 Anemia

Anemia is a condition in which the number of RBCs or their oxygen capacity is insufficient to meet physiological needs(1). Anemia in pregnancy defined as a decreased Hb concentration less than 11gm/dl in venous blood. It is one of the most common nutritional deficiency diseases observed globally and affects more than a quarter of the world's population(9). During pregnancy anemia is considered as severe when Hb concentration is less than 7.0 gm/dl, moderate when Hb falls between 7.0–9.9 gm/dl, and mild when Hb concentration is between 10.0-11 gm/dl(2). It is one of the most worldwide public health problems, especially in pregnant mothers. Nearly half of all pregnant women have been affected by anemia globally. The burden of anemia in pregnant mother living in developing countries is higher (55%) than the developed one (19%)(34). The study conducted in Uganda also found 29.1% of overall prevalence of anemia among pregnant women(35).

Anemia is a significant public health problem in Ethiopia. According to the 2012 Ethiopian Central Statistical Agency report, nationally, 44% of children aged 6-59 months and 17% of women aged 15-49 were anemic(21).. Despite iron supplementation for all pregnant mother in our country, many cross-sectional studies conducted in different part of Ethiopia showed the significant magnitude of anemia among pregnant women; 25.2%, in Aymba, northwest Ethiopia, 56.8%in Gode, western Ethiopia, 61.6% in Bodity, South Ethiopia, 32.8% in Arba Minch, south, Ethiopia and 27.9%.in Harerge, Southeast, Ethiopia(2,9,20,21,24). Globally, anemia contributes for 20% of maternal death(21). Anemia in pregnancy is not only a risk factor of maternal mortality but is also harmful for the fetus due to increased risk of adverse birth outcomes such as intrauterine growth retardation, premature birth and LBW(36).

2.2 Anemia and HP

HP infection continues to be a major public health issue worldwide. The systematic review and meta analysis study showed that the global prevalence of HP infection was 20-50% in developed countries and 50-80% in developing one(29). Regionally the prevalence is; 7-30% in North America, 30-90% in South America, 1.2-70% in Europe, 13-90% in Asia, 50-94%

in the Middle East, 15-20% in Australia, and 48-95% in Africa(30). Another systematic review and meta-analysis conducted in 2017 indicates the global prevalence of HP infection in pregnant women was 46%. High prevalence of HP among pregnant women also reported in developing countries than developed one, such as 25% in Europe, 44% in Asia, 50% in Africa and 62% in South America. The review also showed that the highest prevalence of HP infection in Sudan (94%)(28). The study conducted in Iran and Sudan also showed the prevalence of HP in pregnant women was 54.2% and 69.8%, respectively(31). Cross-sectional study conducted in different part of Ethiopia among the general population showed that the prevalence of HP was; 83.3% in Hawassa, 50.7% in Jinka, 52.4% in Butajera and 71.0 % in Jigjiga(11,14,32,33). A comparative cross-sectional study done in Addis Ababa reported that the prevalence of HP was higher among pregnant women than non pregnant women 33.8 % versus 21.9 % respectively(13). HP is causes not only gastro intestinal disorders but also anemia(25).

HP infection is a potential factor that might play a role in the occurrence of anemia in children and pregnant women. A systematic review and meta-analysis found higher prevalence of IDA in HP infected subjects than uninfected ones(27). The study conducted in Turkey in general population showed that serum hemoglobin level was significantly reduced among individuals infected with HP relative to uninfected patients(37). The cross-sectional prospective study conducted in Butajera showed the prevalence of anemia among HP infected patients was 30.9% and while 22.5% among uninfected patients. This study also showed statistically significant differences in prevalence of anemia among HP infected and uninfected dyspeptic non pregnant patients(14).

The experimental study done in India found higher prevalence of IDA in HP infected pregnant women than uninfected one. This means that the study found lower Hb levels at the beginning of pregnancy in HP infected mothers compared with non infected one and a more unfavorable change in Hb level during course of pregnancy in the infected mothers. Additionally the study conclude that high prevalence of HP infection was seen in pregnant women suffering from IDA and eradication of the infection by triple drug therapy during third trimester enhanced the response to oral iron and folic acid supplementation(25). Another cross-sectional study conducted in Iran among 180 pregnant women showed a significant and adverse correlation between being serologically HP infection and

hemoglobin level. That is the odds of iron deficiency anemia among pregnant women with HP infection was 3.18 times more compared to who were not infected(38).

A cross-sectional study done in Addis Ababa in 2015 showed statistically significant association between HP infection and anemia status among pregnant women(13). The meta analysis study done in 2015 found HP is effective in reducing Hb level among pregnant women and the association was statistically significant(38). Even though many studies found the association of anemia and HP infection, a study done in Sudan in 2014 failed to find the association between HP infection and anemia, and HP infection prevalence was not different in anemic pregnant women compared with non-anemic one(39). The study conducted in Butajera in 2015 in children also was showed the absence of significant different in the prevalence of anemia among HP infected and non infected children(40).

2.3 Anemia and other related factors

2.3.1 Anemia and sociodemographic characteristics

In developing countries, the risk factors of anemia during pregnancy are multi-factorial. This includes nutritional deficiencies, parasitic infection and sociodemographic and economic status of the mothers(9). The study done in Pakistan showed anemia association with educational status of the pregnant women. This study demonstrated that less education was associated with high prevalence of anemia(7). The study done in Bodity found that approximately 26%, 32.4%, 32.4% and 9% of the pregnant women who were in age range of 15-19, 20-24, 25- 29 and 30-34 years were anemic, respectively. It also showed that nearly half of the women with anemia were wives with elementary education(20). The study conducted in Assossa Zone found higher magnitude of anemia among mothers from the larger ($\geq$ 6) family size compared to those from a smaller ($\leq$ 2) family size(16). The cross sectional study conducted in Gode town showed that more than half of the anemic study participants were from large families (>5 family members), married at age 18 years or younger (82.9%), at or below the middle wealth quintile and were illiterate(9). Another study also found associated of anemia with low level of education and rural residence(20). The study conducted in Arba Minch town also found the association of anemia with monthly income of the family (AOR = 4.0; 95% CI: 5.62–11.01) and family size (AOR = 2.8; 95%

CI: 1.17–6.8)(2). The prevalence of anemia in pregnant women in Ethiopia was 22% in 2011 according to the Demographic and Health Survey. It varied by area of residence, with a higher prevalence of anemia in rural women (18%) than urban (11%) and geographical location, with prevalence ranging from 44 % in the Somali region to 9% in Addis Ababa(41). The study done in Harerge found that the prevalence of anemia was higher (34.6%) in pregnant women in the age group of 18-26 years; however, the difference was not significant. It also showed that majority of the study participants were rural residents with significantly (P = 0.001) higher prevalence of anemia compared to their urban counterparts(21). The systematic review and meta analysis done in Ethiopia showed that women living in urban areas were 73% less likely to be anemic during pregnancy than women in the rural area(42).

2.3.2 Anemia, nutritional status and parasitic infection

Pregnancy is considered to be a delightful experience for the expectant mother. Evidences manifested that adequate intake of nutrition is a key component for individual's health and well-being, particularly during pregnancy. It is well documented that inadequate maternal nutrition results in increased risks of Intra Uterine Growth Restriction(IUGR), low birth weight (LBW), preterm birth, prenatal and infant mortality and morbidity(17). An environment of poverty and food shortages results in the increased prevalence of maternal malnutrition, leading to short- and long-term health consequences to both a mother and her child(43). Anemia is one of the most frequent complications related to pregnancy. Half of all the cases of anemia can be attributed to iron deficiency. WHO has reported that iron deficiency is the most prevalent nutritional problem globally, affecting more than 700 million people(15).

The study conducted in northern Ethiopia showed higher prevalence of anemia among pregnant women having a meal frequency of less than 3 times per day were 2 times higher risk of developing anemia as compared to pregnant women who had a meal frequency of more than 3 times/day. It also showed that the prevalence of anemia was higher in pregnant women who did not take iron supplementation during pregnancy as compared to those who were taking iron(44). The likelihood of having anemia was 80% higher among mothers who

did not eat meat in the past one week (prior to the date of survey) compared to those who ate at least once per week [AOR = 1.80, 95 % CI: 1.11, 2.91](16).

The study done in Gode found that iron supplementation during pregnancy and MUAC were significantly associated with anemia in the study population(9). Another study also showed that the risk of developing anemia was also increase in pregnant women with MUAC less than 21 cm and who were not taking iron supplementation during pregnancy(16). But the study conducted in Mekelle was not found the association of anemia with MUAC measurement of the women(45).

Common infections which are chronic and recurrent may impair hematopoiesis and cause anemia. An intestinal parasite and malaria are the leading causes of anemia. Anemia due to deficiency of iron and malaria coexist in malaria endemic regions of the world. Intestinal parasitic like hookworms, schistosomes, trypanosomes, and other infestations may cause blood losses that contribute to anemia(26).

2.3.3 Anemia and obstetric history

During pregnancy, the pregnant mother undergoes significant physiological changes in order to nurture and accommodate the developing fetus. Gravidity, gestational age and inter pregnancy gap were important variables(18). The study done in Assossa zone shown a significant association of anemia with gestational age and IPG. The odds of having anemia were 67% less among mothers found in the third trim ester of pregnancy compared to those found in the first trimester of pregnancy(16). But the study done in Aymba did not observe any relationship between prevalence of anemia and increasing gestational age(46).

The study conducted in Bodity showed found significant association of anemia with gravidity and gestational age of the women(20). Another study conducted in Harerge, Gode and Asosa Zone also found the significant association of anemia with gestational age(9,16,21).

The study done in Arba Minch showed that pregnant women having birth interval less than two years were at higher risk of becoming anemic as compared to those with birth interval more than two years(2). The study done in Asosa zone also showed lower odds of

developing anemia among women with ≥ 2 years of IPG compared to their counterparts(16). Similarly the study conducted in Butajera also found association of anemia with low IPG less than two years(47).

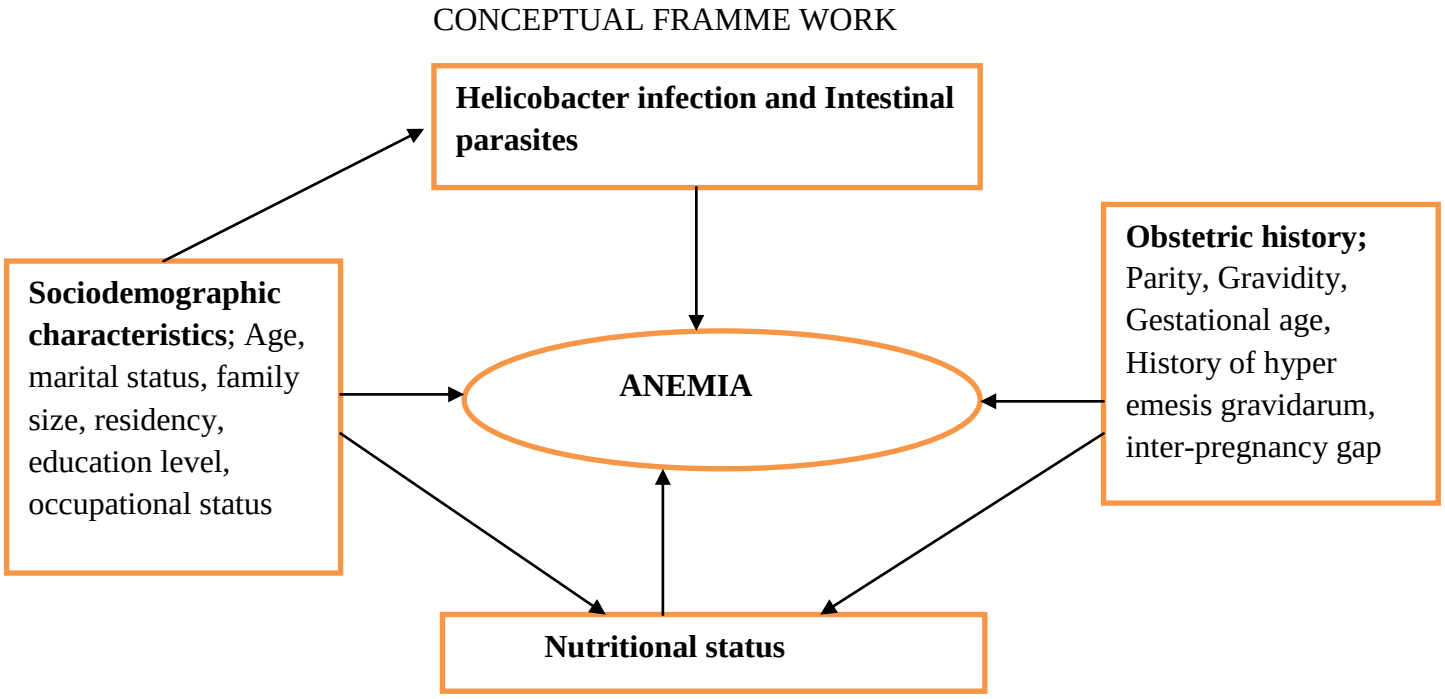

Figure 1 Schematic diagram of conceptual framework (developed) showing the associated and potential risk factors of anemia.

CHAPTER.3. Objectives

3.1. General objective

To investigate the association between anemia and helicobacter pylori infection among pregnant women attending antenatal care in Kulito Health Center, Halaba Special Woreda, Southern Ethiopia, 2018.

3.2. Specific objectives

To investigate the prevalence of anemia among pregnant women

To determine the prevalence of Helicobacter pylori infection among pregnant women

To investigate the association of anemia and Helicobacter pylori infection

To determine the association of anemia and other health related variables

CHAPTER 4 Materials and methods

- **Materials**

Questionnaire was used to collect information on anemia and related risk factors. HemoCue Hb machine with strip, HP serum IgG test strip, slides, slide cover, applicator stick and normal saline were used to test Hb concentration, serum HP IgG status and stool IP infestation. Pen and pencils were used to fill the questionnaire. MUAC tape was used to measure mid upper arm circumference of the study participants to assess their nutritional status.

- **Methods**

4.1 Study Area and period

This study was conducted in Halaba Special Woreda which is found in SNNPR, Ethiopia. Halaba is one of the Special Woreda in SNNPR. It is located about 245 km far from the capital city of Addis Ababa and 90km away in North West direction from the capital of SNNPR, Hawassa. The Special Woreda has 79 rural and 5 urban kebeles with a total population of 323154. In the Woreda there are 10 HCs and two primary hospitals.

The study was conduct in Kulito HC, Halaba Special Woreda, SNNPR, Ethiopia. The HC is among the most patients visited public health institutions in Halaba Special Woreda. The HC serves for 84587 people from 5 urban kebeles and 11 rural kebeles, the HC is delivering service for about 150-200 clients per day, the average pregnant women following antenatal care 20-30 per day. The HC deliver outpatient, ANC and delivery, HIV and tuberculosis treatment, expanded immunization program, family planning with laboratory and pharmacy services.

The study was conducted from April 9 to May 9/2018.

4.2 Study design

Health facility-based cross-sectional study was employed.

4.3 Source population

All pregnant women who have antenatal follow up in Kulito HC ANC clinic are a source population.

4.4 Study population

All pregnant women who have ANC follow up in Kulito HC and attending ANC clinic during the data collection period (April 9 to May 9/2018).

4.5 Inclusion and exclusion criteria:

4.5.1 Inclusion criteria:

All pregnant women who have ANC follow up in the HC during the data collection period were included.

4.5.2 Exclusion criteria:

Women who have active bleeding or history of bleeding during the current pregnancy, have taken treatment for HP in the last two month, who are known to have chronic diseases (heart failure, renal failure, liver disease, diabetic, HIV AIDS, cancer, bleeding disorder) and seriously ill were excluded from the study,

4.6 Sample size determination and sampling techniques

4.6.1 Sample size determination

The sample size was calculated using the general formula for single population proportion sampling. Since there was no previous prevalence (p) of anemia among pregnant women in the study area, but from Butajera 27.6% prevalence of anemia in pregnant women(42). Sample size was calculated by using 27.6% prevalence and adding 10% of non respondent rates.

$$\mathbf{n}= \frac{(z_{\alpha/2})^2 \times pq}{d^2}$$

Sample size = n

Level of significance =0.05

Marginal of error (d) = 5%

Prevalence =27.6%

q= (1-p) =1-0.276= 0.724

Z a/2 = Z-score at 95%

Confidence interval; which is 1.96

Then by using the above formula and information the sample size is 307. By adding 10% non respondent rates, it is 338. The source population is all pregnant women who have antenatal follow up in Kulito HC are 860, which is less than 10,000, and using due correction formula.

$$n_{final} = \frac{n}{1 + \frac{n}{N}}$$

n =338

N=860 n_{final} = 241 which is the final sample size after using correction formula.

4.6.2 Sampling techniques

Study subjects were selected using systematic random sampling technique. In Kulito HC an average of 25 pregnant women attend the ANC clinic each day. The total expected pregnant women during the study period will be estimated to be 750. When the study population was divided by the sample size, the sample interval was 3 and every third pregnant women was select until a total of 241 samples obtained.

4.7 Data collection procedures

The data collectors were two midwives who were assigned in ANC clinic and two laboratory technicians who were working in laboratory department of the HC. They took training for one day on objective of the study, data collection tools and data collection procedures.

4.7.1 Demographic characteristics

Well trained midwives who were working in ANC clinic collected sociodemographic data, water source information, nutritional status and obstetrics history by using questionnaire. The midwives also took measurement of the mid upper arm circumference by using adult MUAC tape to the nearest 0.1 cm.

4.7.2 Blood and stool sample Collection and Processing

Well-trained laboratory technician collected blood and stool specimens in order to ensure that appropriate stool and blood specimen was obtained and Hb concentration, serum IgG status and IP tests were done.

A. Stool Specimen collection and processing

Stool sample was collected in a clean and dry stool cup. Stool sample was collected from all 236 study participants. Fresh stool samples were collected strictly following standard operational procedures with clean stool cup.

Stool sample processing for direct stool examination (wet smear): a drop of normal saline was put on the cleaned microscope slide, a small amount of stool specimen with a wooden stick was taken and mixed with saline on the microscope slide and the sample was examined as soon as possible (within 30 minutes). Helminthes ova was examined using 10x objective and cysts and trophozoites was examined using 40x objectives, this aid to detect certain protozoa trophozoites retain their motility which may aid in their identification(13).

B. Blood specimen collection and processing

A total of 236 blood samples were collected strictly following standard operational procedure of vein blood sample collection procedure, disinfecting the phlebotomy site by swabbing the skin in small outward circles with 70% alcohol swab. Approximately 4 ml of blood was draw from the vein and the blood was mixed properly by inverting the test tube 6-8 times immediately after collection to avoid formation of small clots. Then the samples were tested in laboratory sites to perform Hb concentration and IgG status for HP antibody test. Individuals infected with HP develop antibodies which correlate with histological confirmed HP infection. The HP Antibody Rapid Test Strip (serum) is a qualitative membrane based immunoassay for the detection of HP antibodies in serum or plasma. In this test procedure, anti-human IgG was immobilized in the test line region of the test. After specimen is added to the specimen well of the device, it reacts with HP antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human IgG. If the specimen contains HP antibodies, a colored line was appearing in the test line region indicated a positive result. If the specimen does not contain HP antibodies, a colored line will not appear in this region

indicating a negative result. To serve as a procedural control, a colored line was always appeared in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred(13).

4.8 Study variables:

4.4.1 Dependent variables:-

Anemia

4.4.2 Independent variables:

Helicobacter pylori infection, nutritional status, Parity, gravidity, gestational age, inter pregnancy gap, iron supplementation, age, marital status, family size, residency, education level and occupational status.

4.9 Operational definition

Anemia: venous blood Hb concentration is <11gm/dl (1).

Mild anemia: venous blood Hb concentration is 10-10.9gm/dl(45).

Moderate anemia: venous blood Hb concentration is 7-9.9gm/dl(45).

Severe anemia: venous blood Hb concentration is < 7gm/dl(45).

Primary and above: the context of primary and above educational level expresses the study participants who have attended formal education.

Other health related variables: in this study the context of other health related variables include sociodemographic characteristics, nutritional status, IP infection and obstetrics history of the study participants.

Inter-pregnancy gap: defined as the number of years between the previous live birth and conception of the current pregnancy.

Low inter pregnancy gap: the inter pregnancy gap is less than 2 years.

MUAC is a measurement of the circumference of the upper arm at the midpoint between the olecranon and acromion processes.

MUAC ≥ 21 cm is normal(16).

MUAC < 21 cm is considered as lower (under nutrition)(16).

4.10 Data analysis and interpretation

The collected data was checked for its completeness then coded before entered to SPSS version 21 software. Data were entered into IBM SPSS statistics version 21 (IBM

corporation, USA). Bivarate and multivariate logistic regression models were used to test the association between dependent and independent variables. The dependent variable is anemia status and the independent are sociodemographic characters, nutritional status, HP infections, IP infection, gestational age, IPG and number of pregnancies. In all case a 95% confidence interval was used and P-values less than 0.05 were considered as statistically significant. Results were organized by using frequency tables and charts.

4.11 Data quality management

Data quality was ensured through use of validated questionnaire from other studies with some modifications in local context(13), training was given to data collectors before starting of data collection and strict supervision was followed during data collection by the principal investigator. Questionnaires was translated to Amharic language and then retranslated to English for its consistency by another person and pretest was done in 10% of the sample size of pregnant women who have ANC follow up in Kulito Primary Hospital. The data was checked daily for accuracy, consistency and completeness by the principal investigator and then necessary corrective actions was taken accordingly.

4.12 Ethical consideration

Permission to conduct the study was obtained from the Ethical Committee/Institutional Review Board of Jimma University and letter of cooperation was obtained from Halaba Special Woreda Health Office and Kulito HC Administration prior to the kickoff of the study. The objectives, benefits and risks of the study were explained to the participants and verbal informed consent was obtained from each. The information about the study participants was confidential and those with anemia, HP infections and IP infection were referred to concerned health personnel for appropriate intervention.

4.13 Dissemination plan of results

Thesis report will be submitted to Jimma University School of Graduate Studies, Biomedical Science Department and library. Then the results will submit to Halaba Special Woreda Health Office and Kulito HC. Finally attempts will be made to publish the study result on scientific journal.

CHAPTER 5 Results

5.1 Sociodemographic characteristics of the study participants

Out of the total sample size (241), 236 pregnant women were included with a respondent rate of 97.93%.The mean age of the women was 26.9 ± 6.3(SD) with a range of 16-42 years. Nearly half 109(46.2%) of the women were within the age group 25-32 years. Majority of the study participants were married 222(94.1%) and few of them 14(5.9 %) were divorced. More than half of the participants (59.3%) were urban resident and the remaining (40.7%) were in rural **(Table 1)**.

Table 1 Sociodemographic characteristics of the study participants in Kulito HC, SNNPR, Ethiopia, 2018.

Characteristics		Frequency, N	Percentage, (%)
Age	16-24 years	80	33.9
	25-32 years	109	46.2
	33-42years	47	19.9
Marital status	Married	222	94.1
	Divorced	14	5.9
Educational status	Primary and above	158	66.9
	Illiterate	78	33.1
Occupational status	Employed	99	42
	Unemployed	137	58
Family income	≥ 2000 birr	88	37.3
	< 2000 birr	148	62.7
Family size	≤ 4person	142	60.2
	≥ 5person	94	39.8
Residency	Urban	140	59.3
	Rural	96	40.7
Hand washing before food	Yes	226	95.8
	No	10	4.2
Hand washing after toilet	Yes	201	85.2
	No	35	14.8
Water source	Pipe	214	90.7
	River and others	22	9.3

5.2. Obstetrics history nutritional characteristics and intestinal parasite status of the study participants

Among all pregnant women, 152(64.4%) were multigravida and 84 (35.6%) were primigravida. Majority of the women104 (68.4%) had IPG of more than two years. More than half of the pregnant women 123(52.1%) were in their third trimester followed by those in their second 74(31.4%) and in their first 39(16.5%). Most (93.6%) of the participants had no history of hyperemesis gravidarum during current pregnancy. Some of the study

participants 68(28.8%) were taking iron folate during this pregnancy but majority 168 (71.2%) of them did not take iron folate. More than half 147(62.3%) of the participant had meal ≤ three times a day & 89(37.7%) had four times and above. Nutritional status was evaluated by MUAC and 236 of the respondents were measured, and 44(18.6%) had MUAC of less than 21 cm, and 192(81.4%) had MUAC within normal limits (≥ 21 cm)(Table 2).

Table 2 Obstetrics history and nutritional characteristics of the participants in Kulito HC, SNNPR, Ethiopia, 2018.

Variables	Categories	Frequency, N	Percentage, %
Gravidity	Primigravida	84	35.6
	Multigravida	152	64.4
Gestational age	1st trimester	40	16.5
	2nd trimester	74	31.4
	3rd trimester	132	52.1
Inter pregnancy gap for multigravida	< 2 years	48	31.6
	≥ 2 years	104	68.4
History of hyperemesis gravidarum:-	Yes	15	6.4
	No	221	93.6
Iron pills taking during this pregnancy	Yes	68	28.8
	No	168	71.2
Frequency of diet per day	≥4 times	89	37.7
	≤3 times	147	62.3
Frequency of taking meat at least once a month	Yes	110	46.6
	No	126	53.4
Frequency of taking egg at least once a month	Yes	115	48.7
	No	121	51.3
Frequency of taking fruits at least once a month	Yes	231	97.9
	No	5	2.1
Frequency of taking vegetable at least once a month	Daily	230	97.5
	Every 2 day	6	2.5
Frequency of taking milk and milk product at least once a month	Yes	119	50.4
	No	117	49.6
Taking tea or coffee with food	Yes	220	93.2
	No	16	6.8
Mid upper arm circumference (MUAC)	≥ 21 cm	192	81.4
	< 21 cm	44	18.6
Intestinal parasite	Yes	49	20.2
	No	187	79.8

5.3 prevalence of HP infection

Based on serology IgG test for diagnosis of HP, 129 participants (54.7%) were positive. The burden of anemia was high among illiterate pregnant women 67.9% than educated participants 48.1%.

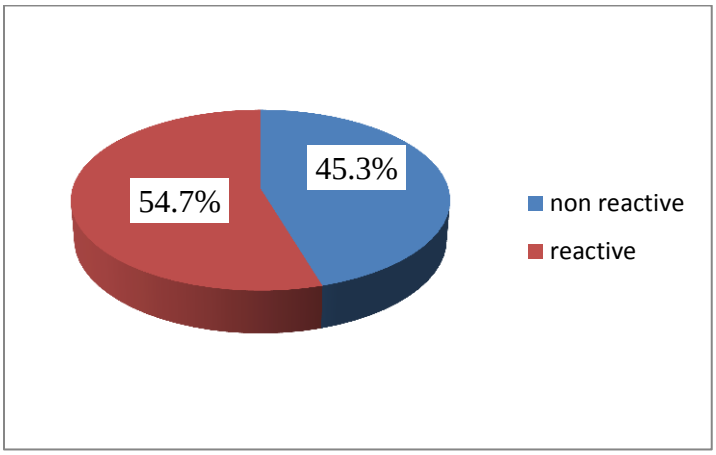

Figure 2. Prevalence of HP infections among pregnant women in Kulito HC, SNNPR, Ethiopia, 2018

5.4 Prevalence of anemia

The participants' Hb level was used to determine the presence or absence and stage of anemia. Hb concentration of the study participants ranged from 7-14gm/dl, with a mean (± SD) of 11.45± 1.58 gm/dl. The overall burden of anemia among the study participants were 65(27.5%) with 15.2% mild, 12.3% of moderate and no severe cases (**Fig 2)**.

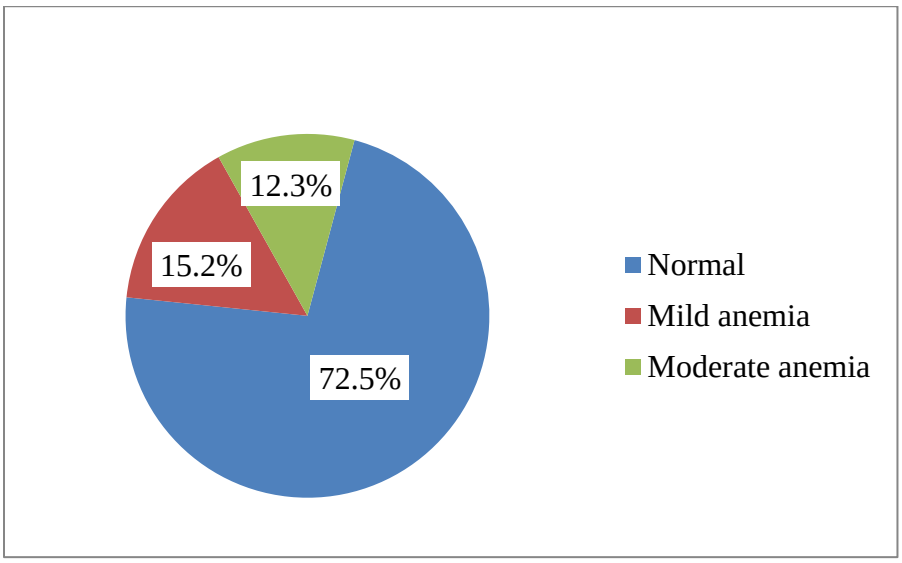

Figure 3 Prevalence of anemia among pregnant women in Kulito HC, SNNPR, Ethiopia, 2018

5.5 Anemia and Helicobacter pylori

The overall prevalence of HP infection among study participants was 129(54.7%).The magnitude of anemia was high among HP infected women than non infected one.

Table 3 Anemia and HP infection among the study participants in a Kulito HC, SNNPR, Ethiopia, 2018.

Variables		Hemoglobin status		Total n (%)
		Anemic n (%)	Normal n (%)	
HP infection	No	18(16.8%)	89(83.2%)	107(45.4)
	Yes	47(36.4%)	82(63.6%)	129(54.6)

HP= Helicobacter pylori

5.6 Anemia and other related factors

The prevalence of anemia was high among pregnant women who were within the age of 32-42 years18/53(43%), unemployed 46/137(33.6%), rural resident 37/96(38.5%) and illiterate 27/78(34.6%). The magnitude of anemia increased among study participants with the family size of more than or equal to five with low monthly family income. The prevalence of anemia was also found to increase as the gestational age increases, showing the highest prevalence in the third trimester (31.5%) compared with second (30%) and first (10.3%) trimester. Women with IPG of less than two years showed more prevalence of anemia (40.8%) than those with an IPG of greater than or equal to two years (22.1%). The prevalence of anemia was high among pregnant women whose MUAC was less than 21 cm (52.3%). The magnitude of anemia was high in women who had IP infection 20/49(40.8%) than non infected one 45/187(24.1%)**(Table 4)**.

Table 4 Anemia and other associated factors among study participants in Kulito HC, SNNPR, Ethiopia, 2018.

Variables		Hemoglobin status		Total n (%)
		Anemic n (%)	Normal n (%)	
Age (years)	16-24	25(31.3)	55 (68.8)	80(33.9)
	24-32	22(20.2)	87 (79.8)	109(46.2)
	32-42	18(38.3)	29 (61.7)	47(19.9)
Occupation	Employed	19(19.2)	80(80.8)	99(42)
	Unemployed	46 (33.6)	91 (66.4)	137(58)
Educational status	Primary& above	38(24)	120(76)	158(67)
	Illiterate	27(34.6)	51(65.4)	78(33)
Family income	≥ 2000 birr	18(20.7)	69(78.3)	87(36.85)
	< 2000 birr	47(31.5)	102(68.5)	149(63.15)
Family size (person)	4 and less	34(23.9)	108(76.1)	142(60.2)
	5 and above	31(33)	63(67)	94(39.8)
Residency	Urban	28 (20)	112 (80)	140(59.3)
	Rural	37 (38.5)	59 (61.5)	96(40.7)
Hand washing after toilet	Yes	50(24.9)	151(75.1)	201(85.2)
	No	15(42.9)	20(57.1)	35(14.8)
Gravidity	3 and less	38 (23.45)	124 (75.55)	162(68.65)
	>= 4	27 (36.5)	47 (63.5)	74(31.35)
Gestational age	1st trimester	4(10.3)	35(89.7)	39(16.5)
	2nd trimester	21(30)	49(70)	70(29.7)
	3rd trimester	40(31.5)	87(68.5)	127(53.8)
IPG(years)	>= 2	23(22.1)	81(77.9)	104(68)
	< 2	20(40.8)	29(59.2)	49(32)
Taking iron folate	Yes	15(22.1)	53(77.9)	68(28.8)
	No	50(29.8)	118(70.2)	168(71.2)
Frequency of meal per day	≥4	26(25.7)	75(74.3)	101(42.8)
	≤3 time	39(28.9)	96(71.1)	135(57.2)
Taking meat at least once a month	Yes	20(18)	91(82)	111(47)
	No	45(36)	80(64)	125(53)
Taking egg at least once a month	Yes	27(23.7)	87(76.3)	114(48.3)
	No	38(31.1)	84(68.9)	122(51.7)
Taking milk at least once a month	Yes	34(28.8)	84(71.2)	118(50)
	No	33(28)	85(72)	118(50)
MUAC in cm	>=21	42(21.9)	150(78.1)	192(81.35)
	< 21	23(52.3)	21(47.7)	44(18.65)
IP infection	No	45(24.1)	142(75.9)	187(79.24)
	Yes	20(40.8)	29(59.2)	49(20.76)

MUAC= mid upper arm circumference, IP= intestinal parasite, IPG= inter pregnancy gap

5.7 Bivarate and multivariate analysis for anemia and related factors

The variables that showed statistically significant association (at p-value ≤ 0.25) in the bivarate analysis were transferred and further analyzed in multivariable logistic regression to adjust for potential confounders and to identify predictors of anemia. In multivariable logistic regression, variables with p-value less than 0.05 were considered as independent factors for anemia. The model was tested for multicollinearity (VIF=1.088-2.022) and Hosmer-Lemeshow test goodness of fit (p=0.810) as a result the model was fit and no multicollinearity exist. In multivariate logistic regression analysis: HP infection, IPG, gestational age and MUAC level were variables independently associated with anemia (Table 5).

Pregnant women with HP infection were 3 times (AOR=3.064, 95% CI: 1.336-7.027) higher to develop anemia as compared to those without HP infection. Another important predictor of anemia was IPG less than two years in which those pregnant women has less than two years of IPG were 2.8 times (AOR=2.863, 95%CI: 1.245-6.582) more likely to suffer from anemia when compared to having IPG ≥ 2 years. Pregnant women who were in the third trimester were 6.5 times (AOR=6.457; 95% CI: 1.276-32.729) higher to develop anemia as compared to those in the first trimester. Most importantly, MUAC level less than 21cm was strongly associated with anemia. Pregnant women with MUAC level less than 21cm were 2.6 times (AOR=2.595, 95%CI: 1.044-6.450) more likely to develop anemia as compared to participants with MUAC level ≥ 21cm.

Table 5 Bivarate and multivariate analysis of anemia and related factors among study participant in a Kulito HC, SNNPR, Ethiopia, 2018.

Variable		Hemoglobin status		COR(95%CI)	AOR(95%CI)
		Anemic n (%)	Normal n (%)		
Age (years)				P= 0.047	P= 0.243
	16-24	25(31.3%)	55 (68.8%)	1	1
	24-32	22(20.2%)	87 (79.8%)	0.556(0.286-1.082)	0.749(0.192-2.921)
	32-42	18(38.3%)	29 (61.7%)	1.366(0.642-2.904)	1.730(0.341-8.772)
Occupation				P= 0.016	P= 0.355
	Employed	19(19.2%)	80(80.8%)	1	1
	Unemployed	46 (33.6%)	91 (66.4%)	2.128(1.153-3.929)	1.514(0.628-3.646)
Educational status				P= 0.089	P= 0.186
	≥Primary	38(24%)	120(76%)	1	1
	Illiterate	27(34.6%)	51(65.4%)	1.672(0.925-3.023)	0.504(0.183-1.391)
Family income				P=0.062	P= 0.586
	≥ 2000 birr	18(20.7%)	69(78.3%)	1	1
	< 2000 birr	47(31.5%)	102(68.5%)	1.810(0.971-3.374)	1.349(0.459-3.963)
Family size (person)				P=0.130	P= 0.459
	≤ 4	34(23.9)	108(76.1)	1	1
	≥ 5	31(33)	63(67)	1.563(0.877-2.785)	0.638(0.194-2.096)
Residency				P=0.002	0.137
	Urban	28 (20%)	112 (80%)	1	1
	Rural	37 (38.5%)	59 (61.5%)	2.508(1.400-4.496)	1.878(0.818-4.310)
Gravidity				P=0.013	0.053
	≤ 3	38 (23.45%)	124 (75.55)	1	1
	≥ 4	27 (36.5%)	47 (63.5)	1.931(1.062-3.511)	2.197(0.990-4.876)
Gestational age				P=0.044	0.06
	1st trimester	4(10.3%)	35(89.7)	1	1
	2nd trimester	21(30%)	49(70)	3.750(1.183-11.889)	3.868(0.696-21.485)
	3rd trimester	40(31.5%)	87(68.5)	4.023(1.339-12.087)	**6.457(1.276-32.729)***
IPG(years)				P=0.018	P=0.013
	≥ 2	23(22.1%)	81(77.9%)	1	1
	< 2	20(40.8%)	29(59.2%)	2.429(1.166-5.061)	**2.863(1.245-6.582)***
Taking iron folate				P=0.232	0.755
	Yes	15(22.1%)	53(77.9%)	1	1
	No	50(29.8%)	118(70.2%)	1.497(0.772-2.902)	1.166(0.444-3.066)
Taking meat at least once a month				P=0.003	0.322
	Yes	20(18%)	91(82%)	1	1
	No	45(36%)	80(64%)	2.500(1.363-4.584)	1.618(0.625-4.194)
Taking egg at least once a month				P=0.174	0.742
	Yes	27(23.7%)	87(76.3%)	1	1
	No	38(31.1%)	84(68.9%)	1.492(0.838-2.658)	0.857(0.342-2.147)
MUAC in cm				P=0.000	P=0.04
	≥ 21	42(21.9%)	150(78.1%)	1	1
	< 21	23(52.3%)	21(47.7%)	3.912(1.975-7.747)	***2.595(1.044-6.450)****
HP infection				P=0.001	P=0.008
	No	18(16.8%)	89(83.2%)	1	1
	Yes	47(36.4%)	82(63.6%)	2.834(1.524-5.271)	***3.064(1.336-7.027)****
IP infection				P=0.021	0.604
	No	45(24.1%)	142(75.9%)	1	1
	Yes	20(40.8%)	29(59.2%)	2.176(1.124-4.235)	1.328(0.454-3.885)

**Statistically significant at 95% CI, p- value < 0.05; 1-reference, CI=confidence interval, AOR= adjusted odds ratio*

CHAPTER 6.Discussion

The burden of prenatal anemia is widely recognized as a major public health problem throughout the world, particularly in developing countries(16). Because of blood volume expansion and increased iron demand of the fetus and the mother, hemoglobin level altered dramatically during the course of pregnancy(8). This study noted that, the prevalence of anemia in this study population was found to be 27.5% (95% CI=22.0, 33.5). It is moderate public health significance according to WHO criterion, which means the magnitude of anemia is within the range of 20-39.9%(49).

The overall prevalence of anemia of this study is comparable with the former studies from various parts in Ethiopia, such as 27.6% from Butajera general hospital, southern Ethiopia(47), 27.9% from Harerge, southeast Ethiopia(21) and 29.1% from Uganda (outside of Ethiopia)(35). The result was slightly higher than the previous local reports from Aymba HC, northwest Ethiopia 25.2%(24). However, this report was lower than another study from such as 61.6% was reported from Bodity, south Ethiopia(20), 56.8% from Gode, southeast Ethiopia(9), 32.8% from Arba Minch, south Ethiopia(2) and 31% from Iran(outside of Ethiopia)(15). Such magnitude differences may be due to differences in inclusion, and exclusion criterion and dietary characteristics of the study participants between the studies. This means, the above study included pregnant women with known chronic illness and bleeding.

Systematic review and meta analysis study found that worldwide prevalence of HP infection in general population ranges from 25% to 94%(50). Another systematic review and meta-analysis conducted in 2017 indicates the global prevalence of HP infection in pregnant women was 46%(28).The prevalence of HP infection in this study population was found to be 54.7% (95% CI: 47.9-61.4).

This prevalence of HP infection is consistent with studies conducted in Jinka(33) and Butajera(14) among general population which reported prevalence of HP infection 50.7% and 52.4% respectively. The result of this study is also similar with the study done in Iran which found prevalence of HP infection: 54.2% among pregnant women(15).

Study conducted among general population in Jigjiga(32) and Hawassa(51) reported high prevalence of HP infection as compared to this study which found prevalence of HP infection to be 71% and 83.3% respectively. However, the result of this study is higher than study conducted among pregnant women in Addis Ababa(13). The difference of the magnitude of HP infection might be due to difference in sample size, socio demography, and especially laboratory methods they used.

Anemia is a problem that is caused by several factors. The present study found IPG as a predictor of anemia and thus, pregnant women with < 2 years of IPG were 3 times more likely to suffer anemia when compared to those having ≥ 2 years of IPG. Similar findings were also reported from the studies conducted at Arba Minch(2), Asosa Zone(16) and Butajera(47). In contrast, the study conducted in Bodity showed that anemia was more prevalent in women with IPG of greater than or equal to two years (87.5%) than those with less than two years (12.5%)(20). Appropriate time after each pregnancy for recovery and replenishment of nutrient stores requires 2–5 years. Pregnancy with a short birth interval leads to IDA as iron requirements are substantially higher than the average(47). The risk of maternal nutritional depletion increases with closed birth intervals. Therefore, mothers need adequate time to restore nutritional reserve until the next pregnancy. Mothers attain good nutritional status, including iron, when there is a gap of at least 2 years between consecutive pregnancies(16).

Gestational age was another factor significantly associated with anemia in this study. Pregnant women in the third trimester of gestational age were 6.5 times more likely to develop anemia as compared to those in the first trimester of gestational. Similarly the study done in Bodity(20), , Harerge(21) and Gode(9) showed that the significant association of anemia with third trimester of gestational age. In contrast the study conducted in Aymba HC, Amhara region and Asosa Zone(16) was failed to find association of anemia with third trimester of gestational age(24).

This could be due to the fact that when the gestational age increases the mother becomes weak and the iron in the blood is shared with the fetus in the womb. Another factors also increasing of total blood volume as the gestational age increases.

Another variable with significant association with anemia was MUAC level. MUAC less than 21 cm is found to increase the risk of developing anemia. The current study showed that pregnant women with MUAC < 21 cm had 2.6 times more risky of developing anemia than those with MUAC ≥ 21 cm. This finding is consistent with a study done in Asosa Zone(16) and Gode(9). However, the study conducted in Mekelle failed to find the significant association of MUAC<21cm and anemia(45). Pregnancy is the most nutritionally demanding time in a woman's life, which increases the vulnerability of mothers for poor micronutrient reserve, including iron. In addition, under nutrition impaired production of iron transport proteins and increased depletion of stored iron which in turn causes anemia(16).

This study also found significant association between anemia and HP infection (AOR = 3.064, 95% CI=1.336-7.027). Pregnant women with HP infection were more likely to have anemia than those without HP infection in agreement with previous reports done among pregnant women in Addis Ababa(13), in Iran(38), India(25), Turkey(37) and another systematic review and Meta analysis(27). Similarly the study conducted in Butajera among dyspeptic non pregnant patient also found significant association between anemia and HP infection(14). However, the study conducted in Butajera among children(40) and Sudan among pregnant women was failed to found association of anemia and HP infection(39).

These association of anemia with HP infection may be due to the possible mechanisms by which HP affects iron metabolism include decreased absorption resulting from chronic gastritis, decreased gastric juice and ascorbic acid secretion due to atrophy of gastric mucosa caused by HP irritation. Ascorbic acid is known to facilitate iron absorption by reduction of iron III to iron II. Increased hepcidin production associated with HP gastritis, uptake of iron by HP for growth, and decreased availability of iron by sequestration of iron in lactoferrin in the gastric mucosa are also the possible mechanisms(27). It has been hypothesized HP infection decreases mucosal iron absorption capacity due to low gastric pH, reduction of stomach vitamin C levels, bacterium-host competition for dietary iron supply; lactoferrin mediated iron sequestration by gastric HP, increased hepatocytes hepcidin release in response to Interlukin-6 production associated with HP gastritis(14).

Limitation of the study

- The cross sectional nature of this study has a limitation to show cause and effect associations of anemia with HP infections and other related factors like IP infection, nutritional status and IPG in pregnant women.
- Another limitation also the determination of HP infection was with only one method by (rapid HP antibody test kit); even though the HP serum antibody test kit employed in this study sensitivity has 97.9%, specificity 97.4% and accuracy 98.9 % (user leaflet of kits).
- Laboratory method used for anemia has limitation to identify the iron status of the mothers.
- Assessing nutritional status only by frequency of different diet could not provide adequate information about the nutritional status of the mothers.

CHAPTER 7 Conclusion and recommendation

7.1. Conclusion

In this study the overall prevalence of anemia among women attending ANC in Kulito Health Center of Kulito town was 27.5% (95% CI=22.0, 33.5). Anemia is a moderate public health problem in Kulito Health Center. The overall prevalence of HP infection in this study population was found to be 54.7% (95% CI: 47.9-61.4). MUAC level, IPG, gestational age of being in third trimester and HP infection was significantly associated with anemia. However, some anemia associated risk factors like not taking iron folate during pregnancy, presence of intestinal parasites, frequency of meal per day and number of pregnancy do not show statistically significant association with anemia.

7.2. Recommendation

In this study the burden of anemia is moderate public health problem according to WHO criterion. We recommend health education on prevention and treatment of HP infection and malnutrition. We also recommend awareness creation on contraceptives usage to widen the inter pregnancy gap.

Further studies are required in the community using different diagnostic methods to explore the actual role of HP infection and I recommend that a large scale study be conducted to further elucidate the role of HP infection and warranted to consider whether screening and treating for HP infection during pregnancy could benefit the mother and the fetus.

References

1. Guyton AC. Guyton and Hall textbook of medical physiology. Thrteenth. Hall JE, editor. 2016. 445 p.

2. Bekele A, Tilahun M, Mekuria A. Prevalence of Anemia and Its Associated Factors among Pregnant Women Attending Antenatal Care in Health Institute of Arba Minch town, Gamo Gofa zone, Ethiopia: A cross sectional study. Hindawi. 2016;2016:1–9.

3. Paulí K, Leyva M, Arocha C, Herrera D. Anemia in Children under Five Years Old in Eastern Cuba , 2005 – 2011. Peer Rewiew. 2014;16(1):16–23.

4. Tadesse E, et al. Determinants of anemia among pregnant mothers attending antenatal care in Dessie town health facilities , northern central Ethiopia , unmatched case - control study. 2017;(Ci):1–9.

5. Francis S. Maternal hemoglobin level and its association with pregnancy outcome among mothers. Nitte Univ J Heal Sci. 2013;3(3):96–100.

6. Sharma B, Shankar M. Anemia in Pregnancy . JIMSA. 2010;23(4):253–60.

7. Shams S. Prevalence of Iron Deficiency Anemia in Pregnant Women of District. J Pregnancy Child Heal. 2017;4(6):1–5.

8. Soma P, Nelson C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr. 2016;27(2):89–94.

9. Kefyalew M. Prevalence of Anemia and Associated Factors among Pregnant Women in an Urban Area of Eastern Ethiopia. Hindawi. 2014;2014(May 2013):1–7.

10. Shatla M. Treatment of Helicobacter Pylori Associated Iron Deficiency Anemia : Does Iron Supplementation Make Difference. Eur J Prev Med. 2016;4(2):50–5.

11. Tadesse E, Daka D, Yemane D, Shimelis T. Seroprevalence of Helicobacter pylori infection and its related risk factors in symptomatic patients in southern Ethiopia. 2014;1–5.

12. Kumar A, Kaushik M. Blood group and anemia : Exploring a new relationship. public

Heal Epidemiol. 2013;5(January):43–5.

13. Terfa K. Prevalence of Helicobacter Pylori infection and associated risk factors among women child bearing ages in selected health centers, Kolfe Keranio sub city, Addis Ababa. Unpublished. 2015;1–54.

14. Kibru D, Gelaw B, Alemu A, Addis Z. Helicobacter pylori infection and its association with anemia among adult dyspeptic patients attending Butajira Hospital , Ethiopia. biomed Cent. 2014;14:1–7.

15. Parashi S, Bahasadri S, Alirezaiei M. Assessing the Association between Iron Deficiency Anemia and H . Py- lori Infection among Pregnant Women referring to a Busy Antenatal Clinic in Tehran-Iran. Shiraz E Med J. 2013;14(3):153–61.

16. Abay A, Yalew HW, Tariku A. Determinants of prenatal anemia in Ethiopia. BioMed Cent. 2017;7:1–10.

17. Daba G, Beyene F, Fekadu H, Garoma W. Nutrition & Food Assessment of Knowledge of Pregnant Mothers on Maternal Nutrition and. Nutr Food Sci. 2013;3(6):1–7.

18. Soma P, Nelson C, Tolppanen A. Physiological changes in pregnancy. Cardiovasc J Afr. 2016;27(2):89–94.

19. Federal Democratic Republic of Ethiopia Demographic and Health Survey Report. Addis Ababa, Ethiopia; 2016.

20. Lelissa D, et al. Prevalence of Anemia Among Women Receiving Antenatal Care at Boditii Health Center , Southern Ethiopia. Clin Med Res. 2015;4(3):79–86.

21. Kefiyalew F, Zemene E, Asres Y, Gedefaw L. Anemia among pregnant women in Southeast Ethiopia : prevalence , severity and associated risk factors. BioMed Cent. 2014;7:1–8.

22. Cardaropoli S, Rolfo A, Todros T. Helicobacter pylori and pregnancy-related disorders Helicobacter pylori and pregnancy-related disorders. 2014;(June).

23. WHO. Guideline : Daily iron and folic acid supplementation in pregnant women. 2012;

24. Asrie F. Prevalence of anemia and its associated factors among pregnant women receiving antenatal care at Aymiba Health Center , northwest Ethiopia. Blood Med. 2017;35–40.

25. Malik R, Guleria K, Kaur I, Sikka M, Radhakrishnan G. Effect of Helicobacter pylori eradication therapy in iron deficiency anaemia of pregnancy – A pilot study. Indian J Med Res. 2011;134(August):224–31.

26. Rahel F. Prevalence of Anemia among Pregnant Women Attending Antenatal Care at Selam Health Center, Addis Ababa, Ethiopis. unpublished. 2016;1–32.

27. Cardaropoli S, Rolfo A, Todros T. Helicobacter pylori and pregnancy-related disorders. World J Gastroenterol. 2014;20(3):654–64.

28. Dehghan R, Cevirme A, Hamlaci Y, Ozdemir K. Global Prevalence of Helicobacter Pylori Infection in Pregnant Women : A Systematic Review and meta-analysis. Int J Women Heal Reprod Sci. 2017;5(1):30–36.

29. Shaweno D and Daka D. Association between O blood group and Helicobacter pylori infection. public Heal Epidemiol. 2017;5(October):471–8.

30. Rana R, Wang L, Wang Y, Rao W, Yang Q. Helicobacter pylori infection : A recent approach to diagnosis and management. 2017;2.

31. Nahla M, Gasim I, Khalifa E. Helicobacter pylori , anemia , iron deficiency and thrombocytopenia among pregnant women at Khartoum , Sudan. Trans R Soc Trop Med hygine. 2014;10(August):1–5.

32. Getachew K. Prevalence of Helicobacter Pylori Infection and Associated Factors among Gastritis Students in Jigjiga University , Jigjiga , Somali Regional State of Ethiopia. Bacteriol Mycol. 2016;3(3):9–12.

33. Hailu G, Desta K, Tadesse F. Autoimmune and Infectious Diseases : Open Access. 2016;

34. Yilikal A. Association of Helicobacter pylori infection and hyperemesis gravidarum women: A case control study in selected Hospital and two health centers in Kirkos Sub-city, Addis Ababa, Ethiopia. Unpublished. 2017;1–57.

35. Baingana RK, Enyaru JK, Tjalsma H, Swinkels DW, Davidsson L. The aetiology of anaemia during pregnancy : a study to evaluate the contribution of iron de fi ciency and common infections in pregnant Ugandan women. Public Health Nutr. 2014;18(8):1423–35.

36. Clinical Guidline. Anaemia in pregnancy, Iron deficiency anaemia, haemoglobin, ferritin, thalassaemia, folic acid, vitamin B12, megaloblastic, microcytic, hypochromic, CBE, iron supplementation, haemoglobinopathy, oral iron, intravenous iron, clinical gui. Vol. 3, SA Health. 2016.

37. Mustafa C, Erim G, Zulfu B SZ. Correlation of anemia and Helicobacter pylori infection among dyspeptic patients in Kutahya region. Eur J Med Sci. 2015;2(1):1–5.

38. Milad Azami, Zahra Darvishi, Milad Borji KS. Helicobacter pylori infection is associated with anemia in pregnant women: a meta analysis study. Iran J Med Microbiol. 2016;10(1):1–7.

39. Al MN et. Helicobacter pylori , anemia , iron deficiency and thrombocytopenia among pregnant women at Khartoum , Sudan. Trans R Soc Trop Med Hyg. 2014;44(August):1–5.

40. Taye B, Enquselassie F, Tsegaye A, Amberbir A, Medhin G, Fogarty A, et al. Effect of early and current Helicobacter pylori infection on the risk of anaemia in 6 . 5-year-old Ethiopian children. BMC. 2015;15:1–12.

41. Zillmer K, Pokharel A, Spielman K, Kershaw M, Ayele K, Kidane Y, et al. Predictors of anemia in pregnant women residing in rural areas of the Oromiya region of Ethiopia. BMC Nutr. 2017;3:1–8.

42. Kassa GM, Muche AA, Berhe AK, Fekadu GA. Prevalence and determinants of anemia among pregnant women in Ethiopia ; a systematic review and meta-analysis. BMC Hematol. 2017;17:1–9.

43. Cambria M. Glosz, Andrew A. Schaffner, Scott K. Reaves MJM and, Papathakis PC. Effect of Nutritional Interventions on Micronutrient Status in Pregnant Malawian Women with Moderate Malnutrition: A Randomized, Controlled Trial. MDPI. 2018;1–14.

44. Gebre A, Mulugeta A. Prevalence of Anemia and Associated Factors among Pregnant Women in North Western Zone of Tigray , Northern Ethiopia : A Cross-Sectional Study. 2015;2015.

45. Mesele M. Prevalence and associated factors of anemia among pregnant women of Mekelle town. BMC Res Notes. 2015;7(February):1–7.

46. Asrie F. Prevalence of anemia and its associated factors among pregnant women receiving antenatal care at Aymiba Health Center , northwest Ethiopia. blood Med. 2017;8:35–40.

47. Getahun W, Belachew T, Wolide AD. Burden and associated factors of anemia among pregnant women attending antenatal care in southern Ethiopia : cross sectional study. BMC Res Notes. 2017;10:1–7.

48. Terfa K. Prevalence of helicobacter pylori and associated factor among women of child bearing age. unpublished. 2015;1–54.

49. WHO repotr. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. WHO report. 2011.

50. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420–9.

51. Birhaneselassie M, Alemneh A. The relationship between dyspepsia and H . pylori infection in Southern Ethiopia. 2017;6(5):86–90.

ANNEXES

Annex I. Informed consent form

Hello dear, my name is---------- I come here as data collector to assess the association of anemia with helicobacter pylori among pregnant women who have ANC follow up in Kulito health center. On this questionnaire your name will not be written and I am going to ask some questions related to Sociodemographic, hygienic practice, obstetric related history, nutritional status and collect blood and stool sample to determine hemoglobin concentration, helicobacter status and intestinal parasite infestation. You may end this interview any time you want. However, it is hoped that your honest answer to these questions will help physicians and policy-makers understand the possible association of anemia in pregnant women. We would greatly appreciate your truthful and active participation in responding to this questionnaire.

Do you agree to participate in the study?

A. Yes B. No

(For data collectors: encircle the choice to show their willingness or unwillingness)

If yes continue the data collection process

Date of interview----------

Interviewer name----------

Signature----------

Annex II: Amharic version of informed consent

የፍቃደኝነት ማረጋገጫ ቅጽ

ጤና ይስጥልኝ,_____እባላለው::እዚህ የመጣሁተ የደም ማነስ ከጨጋራ ባክቴሪያ ጋር የለውን ተዛማጅነት በቁሊቶ ጤና ጣቢያ ለየእርግዝና ክትትል በሚያደርጉ እናቶች ላይ በሚደረግ ጥናት መረጃ ሰብሳቢ ሆኜ ነው፡፡ በዚህ መጠይቅ ስሞ የማይጻፍ ሲሆን ማህበራዊ እናኢኮኖሚያዊ፣ የንጽህና አጠባበቅ ልምድ፣ የአመጋገብ ሁኔታ እና የደም ማነስ ሁኔታ፣ የጨጋራ ባክቴሪያ ሁኔታና የአንጀት ትላትል ሁኔታን ለመመርመር የደመና፡ የሰገራና ሙና የስፈልጋል። እርስዎ በማንኛውመ ሰአት የቃለመጠይቁን ሂደት ማቁአረጥ ይችላሉ። ነገር ግን የእርስዎ ተሳትፎ ለዚ ጥናት ከፍተኛ ዋጋ ያለው ሲሆን በነብሰጡት እናቶች ላይ የሚያደርሰውን ችግር ቀድሞ ለመከላከል ከፍተኛ ሚና አለው።

ለመሳተፍ ፍቃደኛ ነዎት?

ሀ. አዎ ለ. አየደለውም

መልሱ አዎ ከሆነ መጠይቁን ይጀምሩ አይደለም ከሆነ ግን አመስግነው ይሰናበቱ

ቃለ መጠይቁ የተካሄደበት ቀን _____

ቃለመጠይቁን የጠየቀው መረጃ ሰብሳቢ ስም _____

ፊርማ _____

Annex III: English version questionnaires

Facility Name _____year_____

Participant code _____

(For data collector encircle the specific answer for the question below in the table and if there is different answer write it on the space provided).

A. Sociodemographic information (tick) of study participant

s/n	Variables	Answer	
101	Age	1. _____year	
102	Age at marriage in years	1. _____years	
103	Marital status	1. Single 2. Married 3. Divorced 4.Widowed 5. Others specify_____	
104	Level of education	1. Illiterate 2. Primary education 3. Secondary 4. Diploma 5. Higher	
105	Occupational status	1. House wife 2. House wife 3. Farmer 4. House maid 5. Governmental 6. Non governmental	
106	Family income	1. <500birr 2. 500-1000birr 3. 1000-2000birr 4. >2000birr	
107	Number of people in household	1. Two 2. Three 4. Four 5. Five and above	
108	Residence	1. Urban 2. Rural	

B. Some hygienic Applications habits

201	Washing hands before meals?	1. Yes 2. No	
202	Washing hands after toilet?	1. Yes 2. No	
203	Water use for drink (circle one)?	1. Pipe 2. River 3. Pond 4. Others specify_____	

C. Obstetrics History

301	Age at first pregnancy in years	1. _____	
302	Parity	1. Nulliparous 2. Primipara 3. Multipara	
303	Gravidity	1. Primigravida 2. Multigravida	
304	Gestational age in week	1._____ weeks	
305	Inter pregnancy gap in months	1. Primigravida 2. < 2 years 3. >2years	
306	History of Hyper emesis gravidity during this pregnancy	1. Yes 2. No	

D. Nutritional status, Chart review and Laboratory results

401	Getting nutritional education on the importance of taking iron supplementation and nutritional	1. Yes 2. No	

	counseling on extra meal and iron rich food during pregnancy		
402	Iron supplementation	1. Yes 2. No	
403	Number of meals per day	1. One times 2. Two times 3. Three times 4. Other, ____	
404	Frequency of eating meat	1. Daily 2. Every other day 3. Weekly 4. Every two weeks 5. Once a month	
405	Frequency of eating egg	1. Daily 2. Every other day 3. Weekly 4. Every two weeks 5. Once a month	
406	Frequency of eating fruit	1. Daily 2. Every other day 3. Weekly 4. Every two weeks 5. Once a month	
407	Frequency of drinking or/and eating milk and milk products	1. Daily 2. Every other day 3. Weekly 4. Every two weeks 5. Once a month	
408	Frequency of eating green vegetables	1. Daily 2. Every other day 3. Weekly 4. Every two weeks 5. Once a month	
419	Taking tea or coffee immediately before, after or/and with meal	1. Yes 2. No	
410	MUAC in cm	1._____cm	
411	Hemoglobin(HB) or Hematocrit concentration(Hct)	1. Hb_____ or Hct_____	
412	Serum IgG test (serum H. Pylori test)	1. Reactive 2. Non reactive	
413	Stool examination for intestinal parasites	1. Yes 2. No If yes specify A. _____ B. _____ C. _____ D. _____	

Comments_____

Name of principal Investigator _____Date_____

Annex IV: Amharic version of questionnaire

ስም _____

የታካሚው መለያ ቁጥር _____

(ለመረጃ ሰብሳቢው ከዚህ በታች ለሚገኙ ጥያቄዎች ተሳታፊው የመለሰው መልስ ይከበባል ወይም የተለየ መልስ ከመለሱ በተሰጠው ባዶ ቦታ መሙላት ነው)

ክፍልI. ማህበራዊና ዲሞግራፊያዊ ሁኔታን የሚመለከቱጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምላሾች	
101	እድሜ በአመት	1. _____ አመት	
102	በጋቢቻ ወቅተ እድሜ በአመት	1. _____ አመት	
103	የጋቢቻ ሁኔታ	1. ያላገባ 2. ያገባ 3. የተፋታ 4. በሞትየ ተለየ 5. ሌላካለግልጽ	
104	የትምህርት ደረጃ	1. ያልተማረ 2. አንደኛ ደረጃ 3. ሁለተኛ ደረጃ 4. ዲፕሎማ 5. ከፍተኛ የትምህርት ደረጃ	
105	የስራ ሁኔታ	1. የቤት እመቤት 2. አርሶ አደር 3. የጉልበት ሰራተኛ 4. የመንግስት ቅጥረኛ 5. የግል ቅጥረኛ 6. ሌላካለ	
106	የቤተሰብ የገቢ መጠን	1. ‹500ብር 2. 500-1000ብር 3. 1000-2000 ብር 4. ›2000 ብር	
107	የቤተሰብ አባላት ብዛት	1. ሁለት 2. ሶስት 3. አራት 4. አምስት 6. ሌላካለ	
108	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ	

ለ. የተወሰኑየነጽህናትግበራልምድ

201	ሁሌ ከምግብ በፊት እጅን ይታጠባሉ	1. አዎ 2. አለታጠበም	
202	ሁሌ ከሽንት ቤት ቡሃላ እጅን ይታጠባሉ	1. አዎ 2. አለታጠበም	
203	የመጠጥ ውሃ ከየት ነው የሚጠቀሙት	1. ባንባ 2. ወንዝ 3. ምንጭ 4. ሌላካለ	

ሐ. የእርግዝናታሪክ

301	አድሜ በመጀመሪያ እርግዝና በአመት	1. _____	
302	በህይወት የተወለዱ ልጆች ብዛት	1. የመጀመሪያ 2. አንድልጅ 3.ሁለትና ከዚያበላይ _____	
303	ስንተኛ እርግዝናሽ ነው	1. የመጀመሪያ 2.ከዚህ በፊት አርግዛ የምታውቅ ስንተኛ እርግዝናሽ ነው_____	
304	ይህን እርግዝና ካረገዝሽ ስንትኛ ሳምንትሽ ነው	1. _____ ሳምንት	
305	በባለፈው እርግዝናና በዚ እረግዝና መሃል የምን ያህል የጊዜ ርዝማኔ አለ	1. የመጀመሪያ 2. ከሁለት አመት በታች 3. ሁለት አመትና ከዚያ በላይ	
306	በዚህ እርግዝና ወቅት ከፍተኛ የማስመለስ ችግር ገጥሞሽ ነበር	1. አዎ 2. አላጋጠመኝም	

መ. የአመጋገብሁኔታናየላብራቶሪምርመራ

401	በእርግዝና ወቅት መደረግ ስላለበት የአመጋገብ	1. አዎ 2. አላገኘውም	

	ሁኔታ ትምህርት አግኝተሻል		
402	የአይረን ንጥረ ነገር የያዘ እንክብል እየወሰድሽ ነው	1. አዎ 2. አልወስድም	
403	በ 24 ሰአት ዉስጥ ስንቱ ትመገቢያለሽ		
404	ስጋ በምን ያህል ጊዜ ነው የምትመገቢው	1. በፍጹም 2. በየቀኑ 3. በየሁለትቀኑ 4. በየሳምንቱ 5. በየሁለትሳምንቱ 7. በየወሩ 8. ሌላካለ	
405	እንቁላል በምን ያህል ጊዜ ነው የምትመገቢው	1. በፍጹም 2. በየቀኑ 3. በየሁለትቀኑ 4. በየሳምንቱ 5. በየሁለትሳምንቱ 7. በየወሩ 8. ሌላካለ	
406	ፍራፍሬ በምን ያህል ጊዜ ነው የምትመገቢው	1. በፍጹም 2. በየቀኑ 3. በየሁለትቀኑ 4. በየሳምንቱ 5. በየሁለትሳምንቱ 7. በየወሩ 8. ሌላካለ	
407	አትክልት በምን ያህል ጊዜ ነው የምትመገቢው	1. በፍጹም 2. በየቀኑ 3. በየሁለትቀኑ 4. በየሳምንቱ 5. በየሁለትሳምንቱ 7. በየወሩ 8. ሌላካለ	
408	ወተትና የወተት ተዋጽአ በምን ያህል ጊዜ ነው የምትመገቢው	1. ነፍጹም 2. በየቀኑ 3. በየሁለትቀኑ 4. በየሳምንቱ 5. በየሁለትሳምንቱ 7. በየወሩ 8. ሌላካለ	
409	ቡና እና/ወይም ሻይ ከምግብ በፊትእና/ወይም ቡሃላ አዘውትረው ይጠቀማሉ	1. አዎ 2. አልጠቀምም	
410	የላይኛው ክንድ ዙሪያ ልኬት በሴ.ሜ	1. _____ሴ.ሜ	
411	የሄሞግሎቢን ወይም ሄማቶክሪት ውጤት (የደም ማነስ ምርመራ)	1. ሄሞግለቢን _____ግ/ዴሊ 2. ሄማቶክሪት _____%	
412	የጨጉረ ምርመራ ውጤት	1. አለ 2. የለም	
413	የሰገራ ምርመራ ውጤት	1. አለ 2. የለም ካለ አይነታቸውን ዘርእር 1. _____ 2. _____ 3. _____ 4. _____ 5. _____	

DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been fully acknowledged.

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