PREVALENCE AND ASSOCIATED RISK FACTORS OF ANEMIA AMONG NON- PREGNANT WOMEN OF CHILDBEARING AGE IN JIMMA TOWN, SOUTH WEST ETHIOPIA

YAREGAL ASRES (BSc)

A RESEARCH PAPER SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF CLINICAL LABORATORY SCIENCE SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

JANUARY, 2013

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY

PREVALENCE AND ASSOCIATED RISK FACTORS OF ANEMIA AMONG NON- PREGNANT WOMEN OF CHILDBEARING AGE IN JIMMA TOWN, SOUTH WEST ETHIOPIA

BY: YAREGAL ASRES (BSc)

ADVISERS: TILAHUN YEMANE, ASSISTANT PROFESSOR (MD, MSc,)

LEALEM GEDEFAW (BSc, MSc)

JANUARY, 2013

JIMMA UNIVERSITY

ABSTRACT

Background: *Anemia affects one-quarter of the world's population and women are one of the groups it concentrated in, making it a global public health problem. It has a number of impacts in non-pregnant women of childbearing age including reduce working capacity, impaired immunity and beginning pregnancy with depleted iron store and/or hemoglobin concentration.*

Objective: *The aim of this study was to determine prevalence and risk factors of anemia among non-pregnant women of childbearing age in Jimma town, south west Ethiopia.*

Methodology: *A community based cross-sectional study was conducted in Jimma town from June 26 - August 27, 2013. Four hundred forty one representative non-pregnant women of childbearing age were participated in the study. Socio demographic and related data were collected using structured questionnaire. Four ml of venous blood and five gm of stool samples were collected from each study participant. Blood samples were used for complete blood cell count, red cell morphology and hemoparasites. Complete blood cell count was done by CELL-DYN® 1800 (Abbott, USA) for the determination of red cell and hemoglobin parameters. Stool samples were checked for intestinal parasites using both direct wet mount and formol-ether concentration techniques. All descriptive statistics, binary logistic regression and multiple logistic regression analysis were performed using SPSS-V 16 software.*

Result: *The overall prevalence of anemia was 71(16.1%) with mean hemoglobin concentration of 12.96 g/dl (± 1.04) among which 69 were mildly anemic. In morphological types of anemia normocytic normochromic anemia took the highest proportion. Being from 25-36 years old (AOR = 6.53, 95% C.I: 1.82 – 23.39; P = 0.016), lower economic level (AOR = 18.84, 95% C.I: 6.47 – 54.91; P < 0.0001), illiteracy (AOR = 2.16, 95% C.I: 1.67 - 5.18; P= 0.005), multiparty (P < 0.0001), having intestinal parasitic infection (AOR = 3.34 95% C.I: 1.66 - 6.73; P = 0.001), usage of more than two sanitary pads per day during menstruation (AOR = 3.03 95% C.I: 1.43 - 6.41; P = 0.004) and low body mass index (AOR = 4.07, 95% C.I: 1.69 – 9.84; P = 0.002) were found to be risk factors for anemia. But knowledge about anemia and current contraceptive use were identified as they have protective effect.*

Conclusions and recommendations: *In the present study, the prevalence of anemia indicated mild public health importance. It needs a consideration of risk factors identified for prevention and control of anemia among non-pregnant women of childbearing age.*

Key Words: *Anemia, risk factors, non-pregnant women of childbearing age*

ACKNOWLEDGMENT

This paper was developed with intense superintendence of my advisors Dr. Tilahun Yemane (MD, MSc: Assistant Professor) and Mr. Lealem Gedefaw (BSc, MSc). Therefore, I would like to forward my deepest gratitude to them for their constructive comments and scientific guidance.

I would like to express my heartfelt gratitude to Jimma University for financial support to do this thesis. Moreover, my limitless thank goes to those who directly or indirectly involved in this thesis work; especially Mr. Tsegaye Tewolde (BSc, MPH/Epidemiology), Mr. Mulatu Gashaw (BSc, MSc) and Mr. Wondimagegn Addisu (BSc, MSc). My special thanks go to my beloved family especially my wife, Tsehay Hailemaryam for her professional and moral supports at all times in every activities.

Finally, I would like to thank Jimma town health office, data collectors and study participants for their patient and commitment to be involved in this study.

TABLE OF CONTENTS

Contents **page**

LIST OF TABLES

Table **page**

LIST OF FIGURS

Figure **Page**

ABBREVIATIONS

BMI……..……..Body Mass Index

CBC ……. Complete Blood cell Count

CSA ……...……Central Statistical Agency

EDHS…… Ethiopia Demographic and Health Survey

EDTA …….…….… Ethylene Diamine Tetra Acetic acid

HCT …….. Hematocrit

HGB ……..…... Hemoglobin

IDA …… Iron Deficiency Anemia

IPI ……..….Intestinal Parasitic Infection

IUD …… Intrauterine Device

JU ……..…. Jimma University

MCH ……..Mean Corpuscular Hemoglobin

MCHC ……...… Mean Corpuscular Hemoglobin Concentration

MCV …….. Mean Corpuscular Volume

MTB …….. Mycobacterium Tuberculosis

PPW …….Postpartum Women

RBC …… Red Blood Cell

RDW ……..…. Red cell Distribution Width

SOP ……..…….. Standard Operating Procedure

WHO ……World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background

Anemia is a condition characterized by a decrease in the number of red blood cells (RBC's) and/or hemoglobin (HGB), resulting in a lower ability for the blood to carry oxygen to body's physiologic needs. Specific physiologic needs vary with a person's age, gender and residential elevation above sea level. Anemia can be classified by considering morphology of RBCs and its etiology. Based on red cell morphology it is classified in to three as, microcytic and hypochromic like iron deficiency anemia (IDA), normocytic and normochromic like hemolytic anemia; or macrocytic like magaloblastic anemia [1-3].

Anemia is the result of a wide variety of causes that can be isolated, but more often coexist which can be an indicator of both nutrition and health status. The underlying cause of anemia includes nutritional deficiencies as iron deficiency, infections like malaria and intestinal parasitic infection (IPI) and HGB disorder which results either decrease in production or increase in loss of RBCs [2, 4].

Anemia is one of the most common nutritional deficiency disorder observed globally. The most significant contributor to the onset of anemia is iron deficiency so that IDA and anemia are often used synonymously, and the prevalence of anemia has often been used as a proxy for IDA. It is generally assumed that 50% of the cases of anemia are due to iron deficiency [4], but the proportion may vary among population groups and in different areas according to the local conditions. The main risk factors for IDA includes a low intake of iron, poor absorption of iron from diets and period of life when iron requirements are high due to blood loss, growth and pregnancy [4-6].

Although nutritional anemia affects members of both sexes and all age groups, the problem is more prevalent among women and contributes to maternal morbidity and mortality, as well as low birth weight. It has been estimated that nutritional anemia affects almost two-thirds of pregnant women in developing countries. However, many of these women were already anemic at the time of conception in developing countries. In addition to nutritional deficiency there are a number of

factors contributing to the occurrence of anemia in non-pregnant women including blood loss through menstruation and previous delivery [1, 4, 7].

The clinical signs and symptoms of anemia vary depending on the cause of anemia and the speed of onset. Medical history, signs and symptoms, clinical examination and blood tests should ideally be done to diagnose anemia, along with further investigation to establish the underlying cause. However, in many resource-poor settings, in which access to routine biochemical and hematological testing is scarce, diagnosis relies on history and clinical examination alone [4, 8].

Some laboratory based diagnosis of anemia mainly involves hematological parameters including HGB concentration, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC count, hematocrit (HCT), mean corpuscular volume (MCV) and red cell distribution width (RDW). Other tests like serum iron level, total iron binding capacity (TIBC), serum ferritin level, transferine saturation, serum transferrin receptor and other advanced serological and molecular techniques are included for genetic and acquired disorders in HGB to find out underlying cause [2, 3].

Several strategies exist for anemia prevention and control. These includes improvement of dietary intake and food diversification, food fortification with iron and other micronutrients, appropriate disease control and health education [8].The major causes of anemia; iron deficiency, malaria and IPI can be addressed in vulnerable groups using a combination of key interventions, as needed including iron supplements targeted to at-risk groups, fortification of staple foods with iron and other micronutrients for the general population, prevent and treat malaria, and deworming particularly at-risk groups [1, 6, 8, 9].

1.2 Statement of the problem

Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality [6]. It affects one-quarter of the world's population and women are one of the groups it concentrated in, making it a global public health problem [6, 7]. According to World Health Organization (WHO) estimation in 2008, 1.62 billion People were affected, from which 56 million (41.8%) were pregnant women and 468 million (30.2%) were non pregnant women [7].

Anemia is responsible for about 1 million deaths a year globally out of which three-quarters occur in Africa and South-East Asia. According to WHO estimation, the highest prevalence among non-pregnant women of childbearing age was found in Africa 69.9 million (47.5%) and in South-East Asia 182 million (35.7%). It continues to be severe health problem in most countries particularly sub-Saharan countries including Eritrea, Sudan, Djibouti, Kenya and Ethiopia among non-pregnant women of child bearing age [6, 7, 8, 10].

The same report estimated that 7.92 million (52.3%) non-pregnant women of reproductive age were anemic in Ethiopia [7]. A study done in nine administrative regions of Ethiopia on women of reproductive age in 2008 indicates general prevalence of 30.4 %. This study also shows regional burden of anemia as the highest prevalence, 79.4% in Afar region followed by 55.7% in Deri Dewa administration and 32.3% in Oromia region [10]. Many research reports of worldwide, nationwide and even regional level in our country indicates mild to severe public health importance of anemia prevalence regarding to non-pregnant women of childbearing age [6, 10-14].

Anemia is associated with increased risks for maternal and child mortality and constitutes a public health problem in developing countries with different impact [15]. It reduces the work capacity of individuals and entire populations, with serious consequences for the economy and national development. In addition, its negative consequences on the cognitive and physical development of children and physical performance, particularly the work productivity of adults are major concern [4, 15].

In particular the consequences of anemia in non-pregnant women of childbearing age includes reduced work capacity and impaired immunity or high risk of infection directly on them and negative impact on community and/or country development at large. Anemia of non-pregnant women of childbearing age has also a direct impact on anemia of pregnant women as 50% of anemic cases in pregnant women starts at the conception. So that it has a direct consequence in fetal and maternal mortality, premature delivery and low birth weight [1, 4].

Most of the cases anemia is largely preventable and easily treatable if detected in time [16] and at the same time prevention and control of anemia among non-pregnant women of childbearing age is essential to prevent low birth weight and prenatal and maternal mortality [17]. But data on relative contributions of causal factors are limited which makes it difficult to effectively address the problem [6]. The etiology of anemia in Ethiopia is not well established and the information available is limited [18].

Despite the fact that it is largely preventable and easily treatable if detected in time, still continues to be a common cause of mortality and morbidity [16]. Related report also indicated anemia in women of childbearing age is a growing public health problem that justifies the implementation of interventions for its prevention and control should be considered [19].

Beginning pregnancy with non-depleted iron stores is beneficial for the maternal iron status during pregnancy and infant birth weight so that control of anemia in non pregnant women of childbearing age is essential to prevent low birth weight and maternal morbidity and mortality. Cost-effective intervention mechanisms for diagnosis, treatment and prevention are well documented. Effective management of anemia includes treatment of the underlying cause, restoration of HGB concentration to normal levels, and prevention and treatment of complications. However, there are constraints on diagnosis, treatment and prevention of anemia in resource-poor settings of developing countries [20, 21].

Suggested strategies aimed at preventing anemia focused on the major underlying causes in developing countries with the fact that treating and preventing determinants of anemia reduce occurrence and consequences of anemia. But different strategies and intervention mechanisms for treatment and control of underline causes need to know area of focus with magnitude and clear

association of determinants on a certain group of people which is a constraint in developing countries.

Many reports show a variation in prevalence between different continents, countries and regions according to the burden of risk factors associated with anemia on non-pregnant women of childbearing age. This indicates there is a gap to have representative and updated data for the group at which anemia treatment and control intervention to be on the ground, targeted group of people or area of focus. There for the purpose of this study was to determine the prevalence of anemia and its risk factors in non-pregnant women of childbearing age group to minimize these gaps.

CHAPTER-TWO: LITERATURE REVIEW

2.1 Literature review

2.1.1 Prevalence of anemia

According to WHO report of 2008, it is estimated that anemia affects one-quarter of the world's population and is concentrated within preschool age children and women. Global database on anemia, from 1993 to 2005, indicated more than 1.6 billion people, which corresponds to 24.8% of the population were affected by anemia. The greatest numbers of individuals affected were non-pregnant women, 468.4 million with a prevalence of 30.2%. According to this data base, anemia prevalence among non-pregnant women were found as; 47.5% in Africa, 45.7 in South East Asia, 32.4% in Eastern Mediterranean, 21.5% in Western Pacific 19 % in Europe and 17.8 % in America [6, 7].

A report on prevalence of anemia in women of childbearing age for 32 countries; 8 in Central America, 12 in South America, and 12 in the Caribbean; compiled as situational analysis in 2009, indicated the prevalence of anemia among non-pregnant women of childbearing age ranges from 4.8% in Chile to 53.9% in Guyana [9].

A study reported in 2010 regarding to the severity and distribution of anemia among non-pregnant and pregnant women of 15-49 years in India indicated national level of mean HGB concentration for non-pregnant women was 11.78 g/dl; 49.6% of non-pregnant women were anemic (HGB < 12.0 g/dl) and 1.4% are severely anemic (HGB < 7 g/dl) [22]. Similar report on the same group of population, non-pregnant women, in Meghalaya, North East India (2010) indicated 48.8% in which data for the article were derived from the third Indian National Family Health Survey in 2005-2006 [23]. Another study reported in 2001 studied on anemia among non-pregnant women in Bangladesh indicated the prevalence of the group was 73% of which prevalence of mild, moderate and severe anemia were 52%, 20% and 1%, respectively [24]. According to a cross-sectional survey conducted in 2001 on anemia in pregnant, postpartum and non-pregnant women in Lak district, Daklak province of Vietnam reported more postpartum women (PPW) had anemia

(62%) compared to other non-pregnant women (54%). PPW also had more severe anemia (6%) than other non-pregnant women (2.6%) [25]. According to the report of cross-sectional study conducted in Bursa, Turkey (2008) among non-pregnant women of reproductive age, the prevalence of anemia was reported as 32.8% [17].

WHO estimates the highest proportions of individuals affected by anemia are in Africa, 44.4% [6], 47.5% [7] among non pregnant women of reproductive age group. A study based on secondary analysis of data from the demographic and health survey (DHS) carried out in 2001 on multilevel modeling of socio-demographic predictors of various levels of anemia among women in Mali reported 64.2 % of non-pregnant women were anemic of which 43.0%, 18.8 % and 2.4 were mild, moderate and severe anemia respectively [26]. A study in women of reproductive age, parous non-pregnant women, in Dar-es-Salaam, Tanzania in 2002 reported as 49% were anemic of which 1.6% were severely anemic [27].

More than a quarter of Ethiopian women of reproductive age were anemic [11]. In Ethiopia anemia is a severe problem for both pregnant (62.7%) and non-pregnant women of childbearing age (52.3%) [6]. A cross-sectional study conducted in 270 clustered villages drawn from nine administrative regions of Ethiopia (2008) on IDA among women of reproductive age group reported a general anemia prevalence among the group was 30.4 % [10]. Another study done on prevalence of anemia (2010) in Ethiopian women reported 30.4% in all group of women of reproductive age group and 29.8% in non pregnant women [13]. The 2011 Ethiopia Demographic and Health Survey (EDHS) measured HGB levels to identify anemia reported 17% of women age 15-49 were anemic of which 13% have mild, 3% have moderate, and 1% have severe anemia [12]. Although there is moderate variation by urban-rural residence, differences vary greatly by region [10].

2.1.2 Determinant factors

According to the study on prevalence of anemia and its determinants in Ethiopian women, prevalence of anemia was slightly higher among women with parasitic infestation and at the same time anemia was significantly higher in women with history of illness and the association was

retained even when the variable was adjusted for its confounding effect in the logistic regression models signifying that the most probable causes of anemia is nutrition related and to some extent chronic illnesses [13, 14]. Iron deficiency is not the only cause of anemia, but where anemia is prevalent; iron deficiency is usually the most common cause [28]. Intake of vegetables less than once a day and meat less than once a week was common and was associated with increased anemia ($p = 0.001$) [15, 20]. In severely anemic subjects, the HGB concentration decreased further with malnutrition ($P < 0.05$) [29].

According to the study done in Tanzania, 2002, anemia was decreased significantly with increasing body mass index (BMI) ($p=0.042$) [27]. A study done in India, 2003, also reported regarding to BMI as 52% of thin, 50% of normal BMI, and 41% of overweight women were anemic. Prevalence of moderate anemia ranged from 9% for the overweight women to 17% for thin women and for severe anemia from 1% for the overweight women to 4% for the thin women [30]. In under nourished non-pregnant women (low BMI) 55.7% were anemic of which mild anemia 37.3%, moderate anemia 16.2% and severe anemia 2.2% and in normal BMI non-pregnant women 46.7% were anemic of which mild anemia 34.2%, moderate anemia 11.5% and severe anemia 1.9%. The mean HGB levels for low, normal and overweight categories of BMI were 12.6 g/dl (±2.1 g/dl), 12.9 g/dl (±1.9 g/dl) and 13.3 g/dl (±1.9 g/dl), respectively. The difference was statistically significant *($P < 0.0001$)*. Compared to those with normal BMI, women with low BMI were 1.36 times more likely to have anemia [11, 22].

A study done in Bursa, Turkey (2008) among non-pregnant women of reproductive age reported prevalence of anemia as 30.4% and 35.0% among any contraceptive users and none users respectively [17]. A study in women of reproductive age, parous non-pregnant women, in Dar-es-Salaam, Tanzania, (2002) also reported as the prevalence of anemia was significantly lower in women using hormonal contraceptives, compared to non-users; 36% and 54% respectively, ($P = 0.04$) [27]. but another study done in Egypt (1999) on the Prevalence of anemia among clients of family planning clinics in Egypt reported use of intrauterine device (IUD) were significantly associated with the highest prevalence of anemia among all contraceptive users (64.9%), and IUD users had the lowest level of HGB compared to nonusers or users of other methods [31]. one

study in Ethiopia (2011) also reported regarding to contraceptive as those not using contraceptive were 1.4 times ($p = 0.02$) more likely to develop anemia than current contraceptive users [11].

Poor educational status, economic level and high parity are among key factors predisposing women to anemia [11]. According to a report done in India (2008) level of education plays a significant role in the prevalence of anemia in non-pregnant women; illiterate women suffer more from anemia (54.5%) than those who studied up to primary (49.6%), secondary (44.1%), and higher levels (37.7%) [22]. A report in Meghalaya (2010) regarding to educational level indicated no education (47.9%), primary (58.5%), secondary (48.8%), and higher (31%) [23].

Multiparty, young age (20-39 years) and heavy menstrual periods are among risk factors associated with high prevalence of anemia [23, 31]. According to a report on prevalence of anemia among women in Mumbai, India (1998); infertile women and women without living children had the highest HGB values ($p<.01$) as mean HGB values (± SD) were 11.4 ± 1.2 g/dl for women with infertility [32]. A study investigated on severity and distribution of anemia among non-pregnant and pregnant women aged 15 to 49 years in urban and rural sectors of 26 states of India (2008) indicated non-pregnant women aged less than 25 years were most affected by anemia [22]. A report in Meghalaya (2010) regarding to anemia in relation to parity reported as, up to 2 (47.2%) 3-4 (48%) and 5 or above (51.3%) [23]. More than 2 sanitary pads in a day during menstruation ($P=0.000$) and duration of menstrual bleeding more than 5 days ($P=0.000$) were found to be risk factors for anemia [17].

2.2 Conceptual framework

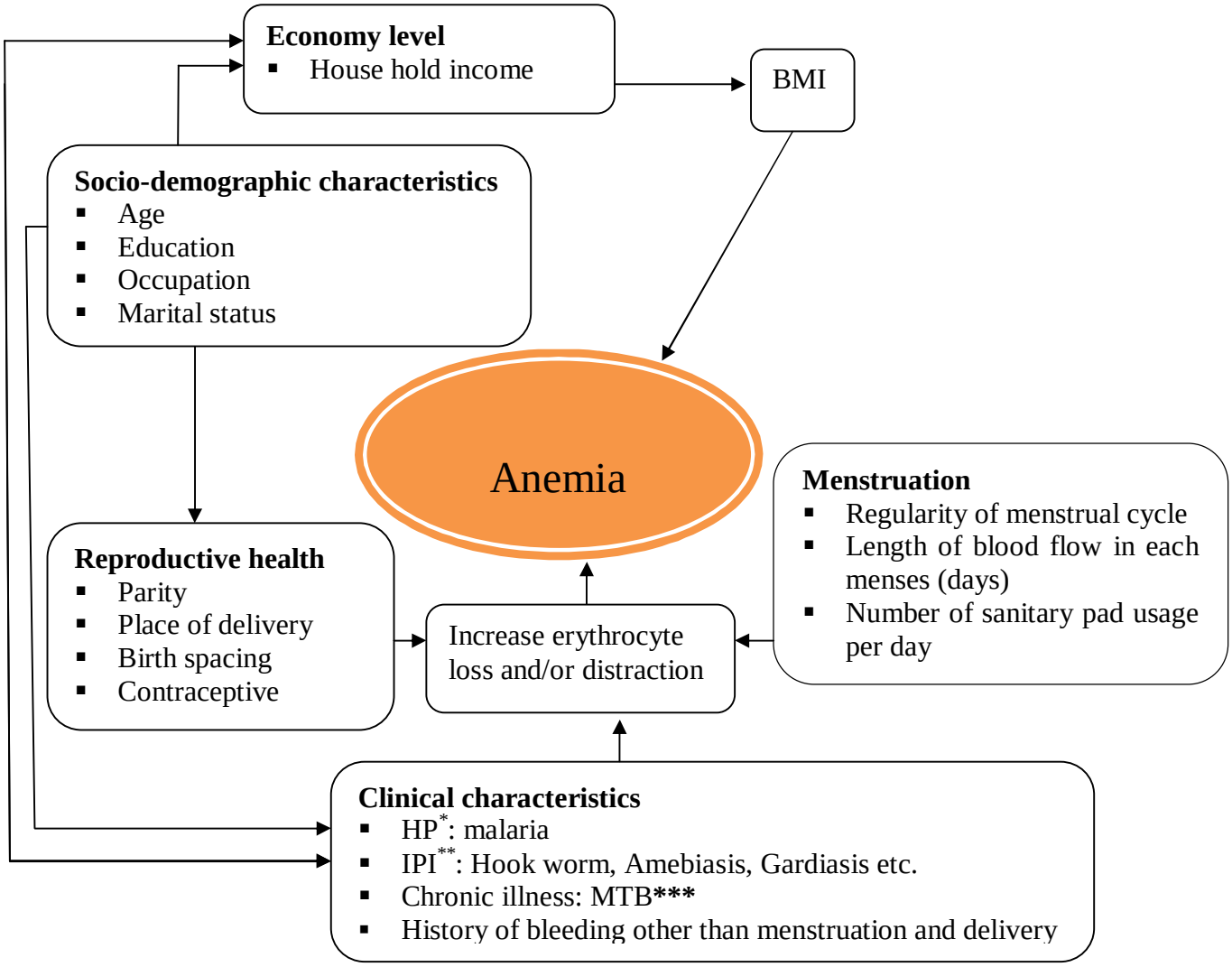

Figure-1- Conceptual framework for determinants of anemia among non-pregnant women of child bearing age in Jimma town, south west Ethiopia, June to August 2013

* = Hemoparasite ** = Intestinal Parasitic Infection ***= Mycobacterium Tuberculosis

The frame work was served as a guide to perform the research tool development and analysis. The information contained in the presentation of conceptual framework were gleaned or adopted from different resources included in the literature reviewed with the consideration of cognitive theory conceptualization of anemia and its determinant factors.

2.3 Significance of the study

Research findings are vital to understand the existing problems and to design strategies of solving those problems, in this case anemia related problems among non-pregnant women of childbearing age. Reduced working capacity, impaired immunity or high risk of infection and learning disability directly on non-pregnant women of childbearing age and indirectly the community and the country at large were identified as some of the outcome of anemia on this group.

An appropriate implementation of anemia prevention and control interventions using different strategies needs current and etiologic relationship with risk factors. Therefore this study may help to determine the prevalence and determinant factors of anemia among non-pregnant women of childbearing age in Jimma town, south western Ethiopia. At the same time, finding of this study will serve as an input for policy makers and local health planners for designing and strengthening proper preventive and control strategies against anemia to reduce its outcome.

In addition the study finding may indicate where focuses should be taken by providing prevalence of anemia and clear associations with its determinants on non-pregnant women of childbearing age. This study will have a contribution on preventing risk of anemia during pregnancy as premature delivery, fetal and maternal mortality and low birth weight with the fact that treating and preventing determinants of anemia reduce occurrence and consequences of anemia.

Ethiopia has planned to lower anemia prevalence in women of childbearing age to 12 % within 5 years, 2010/11 – 2014/15 [33] and the study finding will have an input on plan achievement. In fact the finding of this study will also contribute and add basic knowledge for currently ongoing researches on seeking for association between anemia and determinant factors.

CHAPTER THREE: OBJECTIVES

3.1 General objective:

The aim of this study was to determine the prevalence and associated factors with anemia among non-pregnant women of childbearing age in Jimma Town, south west Ethiopia

3.2 Specific objectives:

- To determine the overall prevalence of anemia among non-pregnant women of child bearing age
- To determine morphological types of anemia among non-pregnant women of childbearing age
- To identify the associated risk factors of anemia among non-pregnant women of childbearing age

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study area and period

The study was conducted in Jimma town, located 350 km south-west of Addis Ababa. The town's geographical locations are approximately 7°41‟ N latitude and 36° 50‟E longitude. The town is found in an area of average altitude of about 1,780m above sea level. It is generally characterized by warm climate with a mean annual maximum and minimum temperature of 30°C and 14°C, respectively. The annual rainfall ranges from 1,138 to 1,690 mm^3. The town is divided in to 13 administrative kebeles (smallest administrative units in Ethiopia) and the total population of the town from 2007 central statistical agency (CSA) census report was 134, 040, of which female accounts 49.7% [34]. It has two governmental hospitals and three health centers providing health care services. There were also health posts in each kebele with health extension workers. The study was conducted from June 26-August 27/2013.

4.2 Study design

A community based cross-sectional study was conducted.

4.3 Population

4.3.1 Source population

All non-pregnant women of child bearing age in Jimma town

4.3.2 Study population

Study population was all selected women in the selected kebeles who fulfills the inclusion criteria.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

To be eligible for participation, woman has to be between 15-49 years old, non-pregnant, permanent resident in Jimma town and voluntary to participate.

4.4.2 Exclusion criteria

Women who had been blood transfused within four months of sample collection, who were on treatments for anemia and PPW less than a period of two weeks were not included as study participant.

4.5 Sample size and sampling method

4.5.1 Sample size determination

The sample size has been determined by considering prevalence of anemia among the group, 17% [12], within 5% marginal error and 95% confidence level. Based on this assumption it was computed using single population proportion formula as indicated below.

$$n = \frac{Z\,(1-\alpha/2)^2 \times P\,(1-p)}{d^2}$$

$$n = \frac{(1.96)^2 \times (0.17)\,(0.83)}{0.05^2}$$

n =217 Where:

n = Sample size

$Z\ \alpha/2$ = Critical value 1.96

P = 17% [12]

d = Degree of precision (marginal error) = 0.05

After multiplying **n** by two for design effect and adding 10% for the anticipated non-response rate, the final sample size was calculated as;

Design effect 2 x 217 = 434

10 % non response rate = 43

Final sample size = 434+43 = **477**

4.5.2 Sampling method

A multistage sampling technique has been employed to select study participants. First, four kebeles were selected from 13 kebeles of the town by simple random sampling method. Selected kebeles were; Hermata, Bochu Bore, Bosa Addis and Seto Semero with their household number of 1,094, 4,987, 2,015 and 2,098 respectively. This is equal to the sum total of 10,194 households with in four study kebeles. Calculated sample size was allocated to selected kebeles proportional to the total household in each kebele. Then proportionally allocated households were selected by systematic random sampling method.

Finally, the study subjects were selected by simple random sampling method from selected households if two or more eligible women to be a study subject were found in a single household. If eligible woman was not found in systematically selected house, the next household has been included. Since there was not enough sample size allocated until the last household, sampling has been continued from the beginning leaving selected household for the first round (Figure-2).

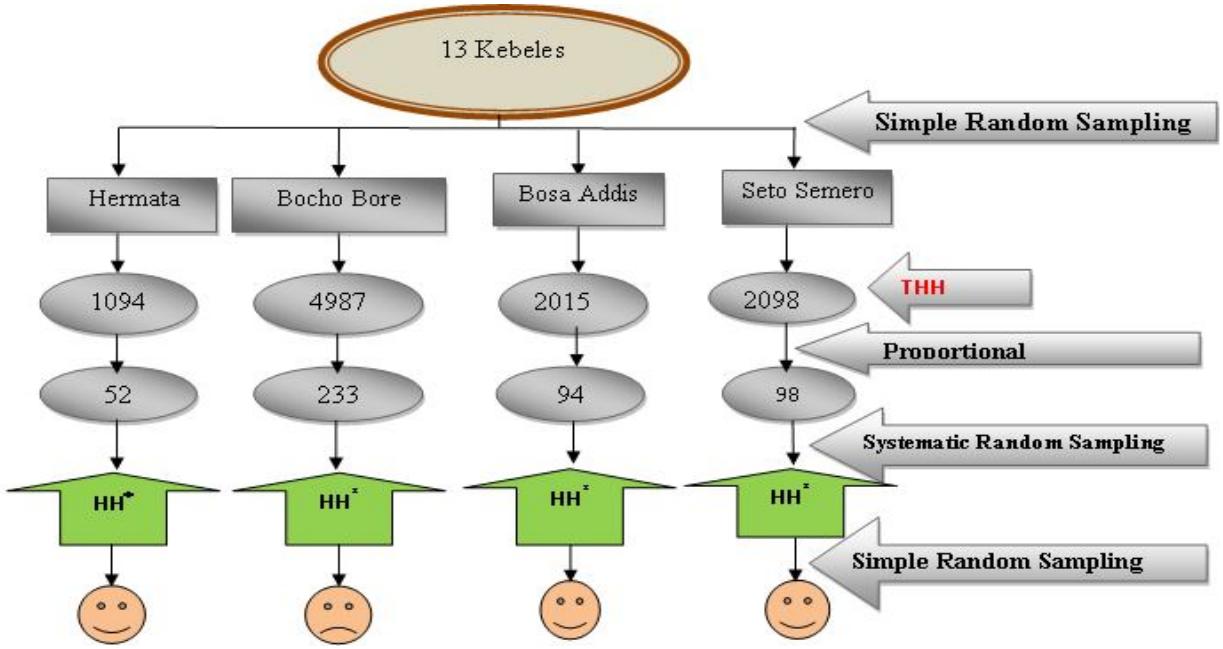

Figure-2- Schematic presentation of sampling techniques on non-pregnant women of child bearing age in Jimma town, south west Ethiopia, June to August 2013

* HH = household = study participant

4.6 Variables

4.6.1 Dependent variable

- Prevalence of anemia

4.6.2Independent variables

- Age
- Educational status
- Marital status
- Occupation
- Household income
- IPI
- Hemoparasites
- Chronic illness, MTB
- Menstruation
- Family planning
- Parity
- BMI

4.7 Data collection techniques and instruments

4.7.1 Socio demographic data collection

Structured questionnaire was developed. The participants then were asked by trained data collector to answer pre-tested questionnaire developed to elicit information on the socio-demographic, socioeconomic and other risk factor related data which were involved in the questionnaire to assess the potential risk factors for anemia (Annex IX).

4.7.2 Blood sample collection and analysis

Four (4) ml of venous blood samples were collected from vein puncture using ethylene diamine tetra acetic acid (EDTA) containing vacutainer test tube after making sure that the study subject sits comfortably and relaxed (Annex III). Then the samples were taken to Jimma University (JU) hematology laboratory within two hours. Then all the laboratory activities were done not later than eight hours of blood sample collection. HGB concentration, MCH, MCHC, RBC count, HCT, MCV and RDW were determined by CELL-DYN® 1800 (Abbott, USA). From the same sample both thick and thin blood films were made (Annex IV) for assessment of hemoparasites and evaluation of red cell morphology (Annex VI). During assessment of red cell morphology, nucleuses of small lymphocytes were used as a reference for their size evaluation and HGB content of the cells were critically observed. The size of RBCs observed on microscope was

assisted by MCV value in automation. Similarly, HGB content of RBCs on microscope was assisted by MCHC and MCH value from automation results.

4.7.3 Fecal sample collection and analysis

Five (5) gm of stool samples were collected from each study participant using clean, wide mouthed and leak proof stool cup. Then stool samples were examined in the nearby health centre within 10-15 minutes of collection by wet mount preparation. Leftover samples were preserved using 10% formalin as a preservative and transported to JU medical parasitology laboratory where they were processed for formol-ether concentration technique (Annex VIII). The types of parasites identified were recorded in the prepared laboratory format in both techniques.

4.7.4 Measurement of weight and height

At the same day of blood and stool sample collection, each study participant was requested to have weight and height measurement by data collector according to the procedure that has been provided for data collector (Annex II).

4.7.5 HCG pregnant test

Women of child bearing age who were not on current contraceptive and at the same time not sure of their pregnancy were requested to give urine sample for human chorionic gonadotropin (HCG) test. It was done following selection of household by systematic random sampling technique but before the consideration of women as a study participant. ADVANCED QUALITY™ one step pregnancy hCG trip tests (InTeC®) were used for the qualitative detection of HCG (Annex I). Women who were negative for the tests were included in the study but positive were not.

4.8 Operational definitions

- **Women of reproductive age:** All women in the age group of 15-49 years old.
- **Hypochromia:** MCHC value < 32 g/dl and increase central pallor area of RBCs on microscopic examination
- **Microcytic:** MCV value < 80 fl and RBCs size smaller than small lymphocyte nuclease on microscopic examination

- **Mild anemia:** HGB concentration HGB concentration in the study participants as measured in venous blood sample by CELL-DYN® 1800 between 10g/dl and 11.9g/dl
- **Moderate anemia**: HGB concentration in the study participants as measured in venous blood sample by CELL-DYN® 1800 between7g/dl and 9.9 g /dl
- **Severe anemia**: HGB concentration HGB concentration in the study participants as measured in venous blood sample by CELL-DYN® 1800 less than 7 g/dl
- **Severe or high public health significance**: Prevalence of anemia ≥40% among non-pregnant women of childbearing age
- **Moderate public health significance:** Prevalence of anemia 20-39% among non-pregnant women of childbearing age
- **Mild public health significance:** Prevalence of anemia 5-19% among non-pregnant women of childbearing age
- **Economy level:** Monthly household income in Ethiopian birr

4.9 Data processing and analysis

All data from the laboratory report forms (Annex XV) and all the data from the questionnaires (Annex IX) were checked for completeness and edited for inconsistencies. Then all the data were coded, entered, cleaned and analyzed by using SPSS-V.16 software.

Descriptive statistics were used to give a clear picture of background variables like age and to determine the prevalence of anemia. Binary logistic regressions were used to identify the association between independent and outcome variables. Multiple logistic regressions were conducted to identify independent predictors of anemia. All explanatory variables that have been associated with the outcome variable in bivariate analyses at 25% level of significance were entered into backward multiple logistic regression model and then variables significant at P-value < 0.05 were identified as independent predictors from the final model.

During analysis, 95 % confidence interval and 5% level of precision were utilized to check for association between variables by considering P-value less than 0.05 as statistically significant. Finally, the data were described and presented using tables and graphs.

4.10 Data quality assurance

Prior to data collection the data collectors were trained for two days by both principal investigator and research coordinator on how to collect data. The questionnaire was translated to the local language, Amharic and Afan Oromo, and back to English version. A pretest was conducted in 5% of the total sample size by trained data collectors for the questionnaire. Ambiguous questions and repetitive ideas were corrected. Pre test was performed in one of 13 kebeles called Ginjo Gudru, other than four kebeles which have been selected for the study and the data were not included.

Up on data collection any unclear ideas and terms were explained well for the respondents in local language. During data collection process, supervisor and principal investigator were checked the questionnaires for completeness and any incomplete or misfiled questionnaires were sent back to the respective data collector for correction.

For better quality of the laboratory results, EDTA anti coagulated vein puncture blood samples were collected following SOP (Annex III). Blood samples were rejected when there were hemolyzed and/or clotted, tubes not filled with minimum volume, improperly labeled and corrective actions were taken.

Similarly, fresh and sufficient quantities of stool samples were collected by providing appropriate instructions to the subjects. The participants were instructed to scoop a two thumbs size (5gm) fecal sample using a provided scoop into the container, making sure that the sample was not contaminated. Stool samples were processed and examined in both direct microscopy and formol-ether concentration techniques following the corresponding SOPs (Annex VIII). To ensure accuracy, 5% of the positive and negative samples were re- examined by other laboratory technologists, who has been blinded to the diagnosis of the first reader.

All laboratory activities were performed strictly following manufacturers' instruction and specific SOPs including quality control materials accordingly. The results of laboratory examination were recorded on well standardized report format carefully according to subject's unique identification number.

4.11 Ethical considerations

Ethical approval was obtained first from the ethical review board committee of JU. Then a written support letter was obtained from the department of medical laboratory science and pathology and given to Jimma town health office. Prior to data collection, permission was obtained from Jimma town health office by providing official letter for health centers. Before the commencement of the study, brief explanations about the objectives of the study were given and voluntary written informed consent was taken from each participant. Parents or guardians oral assent for study participants less than 18 years old were taken. The study participants were involved voluntarily without any cost prerequisite and they were told to have the right not to involve in the study.

To insure the confidentiality, the data collected and results of laboratory tests were used only for this research. Participants with anemia, IPI and malaria were treated by the appropriate treatments under consultation of physicians. In addition, health professionals were provided health education for each study subjects regarding to prevention and controlling mechanisms of anemia.

4.12 Dissemination plan and use of the result

The result of the study will be submitted to JU College of public health and medical sciences, department of medical laboratory science and pathology. It will also be presented to JU community as part of MSc thesis defense and it will be disseminated to the regional health bureau, zonal health office and Jimma town health office. Publish it on local or international scientific journals.

CHAPTER FIVE: RESULTS

5.1 Socio demographic and clinical characteristics of study participants

Out of a total sample size (477), 441 non-pregnant women of childbearing age were enrolled in the study with response rate of 92.45 %. Study participants were mainly within the age group of 25-35 years old (46%) followed by 15-24 (32.0%) with mean (± SD) age of 28.9 ± 8.6 years. Majority of study participants 358 (81.2%) were married. From the total respondents, majority 155 (35.1%) were housewives.

The family size of study participants were ranged from 1 to 9 with an average of 4.28 persons per household. Majority of households 350 (79.4%) were with family size of 1-5 and majority 216 (49%) respondents had > 1000 Ethiopian birr per month per household.

Among all study participants, 10 (2.3%) had history of chronic illness, mycobacterium tuberculosis (MTB). Regarding to knowledge and practice on anemia, among all study participants 179 (40.6%) were heard about anemia. All 441 study participants' blood and stool samples were checked for hemoparasites and intestinal parasites respectively. Accordingly, 5 (1.1%) were confirmed as they had malaria and 147 (33.3%) had at least one intestinal parasite (Table 1).

Table-1- Socio-demographic and clinical characteristics among non-pregnant women of childbearing age in Jimma town, south west Ethiopia, June to August 2013 **(n = 441)**

Variable	Categories	Frequency (%)
Age	15-24	141 (32.0)
	25-35	203 (46.0)
	36-49	97 (22.0)
Marital status	unmarried	83 (18.8)
	Married	358 (81.2)
Educational status	Illiterate	132 (30.0)
	Grade 1-8	160 (36.3)
	Grade 9 and above	149 (33.7)
Occupation	Employee	119 (27.0)
	Housewife	155 (35.1)
	daily laborer	97 (22.0)
	Student	59 (13.4)
	Others	11 (2.5)
Family size	1-5	350 (79.4)
	>5	91 (20.6)
Monthly household income	<500	63 (14.3)
	500-1000	162 (36.7)
	>1000	216 (49.0)
History of chronic illness, MTB	yes	10 (2.3)
	No	431 (97.7)
History of bleeding other than delivery and menstruation	yes	45 (10.2)
	No	396 (89.8)
Ever heard about anemia	Yes	179 (40.6)
	No	262 (59.4)
Presence of current malaria	Yes	5 (1.1)
	No	436 (98.9)
Presence of current IPI	Yes	147 (33.3)
	No	294 (66.7)

MTB = Mycobacterium Tuberculosis, IPI = Intestinal Parasitic Infection

5.2. Reproductive health and nutrition related characteristics of study participants

Majority of study participants participants 264 (59.9%) were currently use contraceptive methods. Regarding to the types of contraceptive, most of contraceptive users 156 (59.1%) were used deppo provera followed by pills, implant and IUD with corresponding number of respondents 51 (19.3%), 48 (18.2%) and 9 (3.4%) respectively. Concerning menstruation related characteristics of respondents, majority 278(63%) had regular menstrual cycle. Most of the study participants 390 (88.4%) had BMI ≥ 18.5 (Table 2).

Table-2- Reproductive health and nutrition related characteristics of non-pregnant women of childbearing age in Jimma town, south west Ethiopia, June to August 2013

Variable	Categories	Frequency (%)
Children ever born **(n = 441)**	No child	110 (24.9)
	1-2	154 (34.9)
	3-5	152 (34.5)
	> 5	25 (5.7)
Place of delivery in health institution * **(n = 331)**	Yes	208 (62.8)
	No	123 (37.2)
Status of current breast feeding **(n = 331)**	yes	108 (32.6)
	No	223 (67.4)
Birth spacing ** **(n = 259)**	≤ 2	118 (45.6)
	> 2	141 (54.4)
Current contraceptive use **(n = 441)**	Yes	264 (59.9)
	No	177 (40.1)
Regularity of menstrual cycle **(n = 441)**	Regular	278 (63.0)
	Irregular	163 (37.0)
Length of blood flow in each menses (days) **(n = 441)**	1-5	387 (87.8)
	≥ 6	54 (12.2)
Number of sanitary pad usage per day **(n = 441)**	1-2	334 (75.7)
	≥ 3	107 (24.3)
BMI **(n = 441)**	< 18.5	51 (11.6)
	≥ 18.5	390 (88.4)

BMI = Body Mass Index, * = Place of delivery service in health institution during the recent birth, ** = Birth spacing between the recent two consecutive children

5.3 Prevalence and types of anemia

The mean ± SD results of were 12.96 ± 1.04 g/dl for HGB concentration, 28.36 ± 1.60pg for MCH, 32.15 ± 1.21 g/dl for MCHC, 4.58±0.37 x 10^{12}/l for RBC count, 40.31 ± 3.09 % for HCT, 88.23 ± 4.5 fl for MCV and 13.99 ± 1.38% for RDW. The prevalence of anemia in this study was 71 (16.1%). 69 (15.6%) and 63 (14.3%) were mildly anemic and had normocytic normochromic anemia (Figure-3).

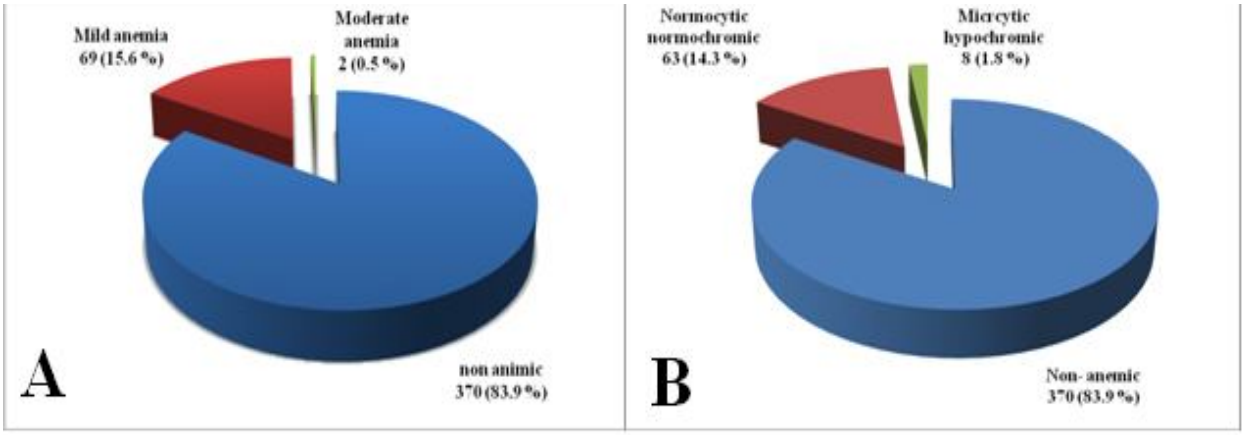

Figure-3- Severity (A) and morphological types (B) of anemia among non-pregnant women of childbearing age in Jimma town, south west Ethiopia, June to August 2013

Among study participants with the age group 25-35, 40 (19.7%) were anemic while 10 (10.3%) were anemic among respondents with the age group of 36-49 (P = 0.111). Among study participants who had < 500 Ethiopian birr household income per month, 23 (36.5%) were anemic while only 8 (3.7 %) of respondents who had > 1000 Ethiopian birr household income per month were anemic (P < 0.0001) (Table 3).

Regarding to current IPI, among study participants who had current IPI, 45 (30.6%) were anemic (P < 0.0001). As intestinal parasites per individual is concerned, a single parasitic infection 137 (93.2%) was more prevalent than multiple parasitic infections 10 (6.8%). A total of nine types of intestinal parasites were identified among which *Ascaris lumbricoides* 59 (40.1%) took the highest proportion followed by *Giardia lamblia* 26 (17.7%), *Trichuris trichiura* 19 (12.9%), *Entamoeba histolytica*/*dispar17* (11.6%), *Hookworm* 13 (8.8%), Stronglyoides *stercoralis* 7 (4.8%), *Schistosoma mansoni* 6 (4.1%), *Enterobius vermicularis* 6 (4.1%) and *Hymenolepis nana* 4 (2.7%).

Table-3- Association of anemia with socio-demographic and clinical factors among non-pregnant women of childbearing age in Jimma town, south west Ethiopia, June to August 2013 **(n = 441)**

Variable	Categories	ANEMIA No (%) Yes	No	COR (95% C.I)	P-values
Age	15-24	21(14.9)	120 (85.1)	1.522 (.68, 3.39)	
	25-35	40 (19.7)	163 (80.3)	2.14 (1.02, 4.48)	0.111
	36-49	10 (10.3)	87 (89.7)	1	
Marital status	Unmarried.	6 (7.2)	77 (92.8)	1	0.019
	Married	65 (18.2)	293 (81.8)	2.85 (2.19, 6.82)	
Educational status	Illiterate	34 (25.8)	98 (74.2)	2.69 (1.42, 5.1)	
	Grade 1-8	20 (12.5)	140 (87.5)	1.11 (0.56, 2.21)	
	Grade 9 and above	17 (11.4)	132 (88.6)	1	0.002
Occupation	Employee	12 (10.0)	107 (90.0)	1	0.001
	Housewife	22 (14.2)	133 (85.8)	1.48 (0.7, 3.12)	
	daily laborer	28 (28.9)	69 (71.1)	3.62 (1.73, 7.59)	
	Student	5 (8.5)	54 (91.5)	0.83 (0.28, 2.46)	
	Others	4 (36.4)	7 (63.6)	5.1 (1.3, 19.67)	
Family size	1-5	56 (16.0)	294 (84.0)	1	0.911
	> 5	15 (16.5)	76 (83.5)	1.04 (0.56, 1.93)	
Household income	< 500	23 (36.5)	40 (63.5)	14.9 (6.25, 35.78)	
	500-1000	40 (24.7)	122 (75.3)	8.53 (3.86, 18.80)	
	>1000	8 (3.7)	208 (96.3)	1	0.0001
History of chronic illness, MTB	Yes	7 (70)	3 (30)	13.38 (3.37, 35.1)	
	No	64 (14.8)	367 (85.2)	1	0.0001
History of bleeding*	Yes	16 (35.6)	29 (64.4)	3.42 (1.74, 6.71)	
	No	55 (13.9)	341 (86.1)	1	0.0001
Ever heard about anemia	Yes	15 (8.4)	164 (91.6)	1	0.0001
	No	56 (21.4)	206 (78.6)	2.97 (1.62, 5.45)	
Presence of current malaria	Yes	3 (60.0)	2 (40.0)	8.12 (1.33, 49.49)	
	No	69 (15.8)	367 (84.5)	1	0.023
Presence of current IPI	Yes	45 (30.6)	102 (69.4)	4.55 (2.67, 7.76)	
	No	26 (8.8)	268 (91.2)	1	0.0001

COR = Crude Odds Ratio, C.I = Confidence Interval, MTB = Mycobacterium Tuberculosis, IPI = Intestinal Parasitic Infection, * = History of bleeding other than menstruation and delivery

Regarding to reproductive health related factors, among study participants who have no children ever born 6 (5.5%) were anemic (P = 0.013). From study participants who had length of blood flow ≥ 6 days in each menses, 19 (35.2%) had anemia (P < 0.001) and 33 (30.8%) of respondents who have used ≥ 3 sanitary pad per day were anemic (P < 0.001). among respondents who have no current contraceptive users, 37 (20.9%) were anemic (P < 0.026) and 24 (47.1%) among respondents with BMI < 18.5 (P < 0.001) were anemic (Table 4).

Table-4- Association of anemia with reproductive health and nutrition related factors among non-pregnant women of childbearing age in Jimma town, south west Ethiopia, June to August 2013

Variable	**Categories**	**Anemia, No (%)**		**COR (95% C.I)**	**P-values**
		Yes	**No**		
Children ever born **(n = 441)**	No child	6 (5.5)	104 (94.5)	1	.013
	1-2	29 (18.8)	125 (81.1)	4.02 (1.60, 10.06)	
	3-5	31 (20.4)	121 (79.9)	4.44 (1.78, 11.06)	
	>5	5 (20.0)	20 (80.0)	4.33 (1.21, 15.58)	
Delivery in health institution *** (n = 331)**	Yes	27 (13.0)	181 (87.0)	1	0.0001
	No	38 (30.9)	85 (69.1)	2.99 (1.72, 5.23)	
State of current breast feeding **(n = 331)**	Yes	28 (25.9)	80 (74.1)	1.76 (1.01, 3.07)	
	No	37 (16.6)	186 (83.4)	1	0.047
Birth spacing **** (n = 259**)	≤2	39 (33.1)	79 (66.9)	5.31 (2.62, 10.74)	
	>2	12 (8.5)	129 (91.5)	1	0.0001
Current contraceptive use **(n = 441)**	Yes	34 (12.9)	230 (87.1)	1	0.026
	No	37 (20.9)	140 (79.1)	1.79 (1.07, 2.98)	
Regularity of menstrual cycle **(n = 441)**	Regular	51 (18.3)	227 (81.7)	1.61 (0.92, 2.81)	0.092
	Irregular	20 (12.3)	143 (87.7)	1	
Length of blood flow in each menses **(n = 441)**	1-5	52 (13.4)	335 (86.7)	1	0.0001
	≥6	19 (35.2)	35 (64.8)	3.5 (1.86, 6.57)	
Number of sanitary pad usage per day **(n = 441)**	1-2	38 (11.4)	296 (88.6)	1	0.0001
	≥3	33 (30.8)	74 (69.2)	3.47 (2.04, 5.91)	
BMI **(n = 441)**	<18.5	24 (47.1)	27 (52.9)	6.49 (3.45, 12.16)	0.0001
	≥18.5	47 (12.5)	343 (87.5)	1	

COR = Crude Odds Ratio, C.I = Confidence Interval, BMI = Body Mass Index, * = Place of delivery service in health institution during the recent birth, ** = Birth spacing between the recent two consecutive children

5.4 Independent predictors of anemia

Multiple logistic regression analysis was performed to identify the risk factors or independent predictors of anemia among non-pregnant women of childbearing age. Thirteen explanatory variables that have been associated with anemia in bivariate analyses at 25% level of significance were entered into backward multiple logistic regression model.

After controlling those factors, in the last step of analysis three variables named marital status, regularity of menstrual cycle and length of blood flow in each menses in days were removed from the final model. The other ten variables were found to be predictors with their corresponding likelihood ratio (Table 5).

According to this analysis age group of 25 - 35 years old (AOR = 6.53, 95% C.I: 1.82 – 23.39) were found to be more anemic compared to age group of 36 - 49 years old. Illiterate non-pregnant women of childbearing age were 2.16 times more likely to be anemic (AOR = 2.16, 955 C.I: 1.67 – 5.18) compared to the same group of women who have learned grade nine and above. Similarly, household income < 500 (AOR = 13.97, 95% C.I: 5.06 - 38.58) and 500 - 1,000 Ethiopian birr (AOR = 4.98, 95% C.I: 2.04 - 12.19) were more anemic compared to those who had > 1000 household income of Ethiopian birr per month. Assessment was also indicated that history of bleeding other than delivery and menstruation (AOR = 2.632, 95% C.I: 1.01 - 6.88, current presence of IPI (AOR = 3.05, 95% C.I: 1.54 - 6.05), no current use of contraceptive (AOR = 4.48, 95% C.I: 2.10 - 9.57), use of sanitary pad ≥ 3 per day (AOR = 2.72, 95% C.I: 1.31 - 5.64) and BMI < 18.5 (AOR = 3.25, 95% C.I: 1.39 - 7.60) showed significant association with anemia compared to respective category (Table 5).

Table-5- Independent predictors of anemia from multivariate logistic regression model among non-pregnant women of childbearing age, Jimma town, south west Ethiopia, June to August 2013 **(n = 441)**

Variable	Categories	COR (95% C.I)	P-value	AOR (95% C.I)	P-value
Age	15-24	1.522 (.68, 3.39)		2.42 (0.92, 6.37)	
	25-35	2.14 (1.02, 4.48)		6.53 (1.82, 23.39)	
	36-49	1	0.111	1	0.016
Educational status	Illiterate	2.69 (1.42, 5.1)		2.16 (1.67, 5.18)	
	Grade 1-8	1.11 (0.56, 2.21)		1.4 (.15, 1.04)	
	Grade 9 and above	1	0.002	1	0.005
Household income	< 500	14.9 (6.25, 35.78)		18.84 (6.47, 54.91)	
	500-1000	8.53 (3.86, 18.80)	0.0001	5.9 (2.31, 15.09)	
	> 1000	1		1	0.0001
History of bleeding*	Yes	3.42 (1.74, 6.71)		3.28 (1.24, 8.68)	
	No	1	0.0001	1	0.017
Ever heard about anemia	Yes	1	0.0001	1	0. 003
	No	2.97 (1.62, 5.45)		3.33 (1.5, 7.42)	
Presence of current IPI	yes	4.55 (2.67, 7.76)		3.34 (1.66, 6.73)	
	No	1	0.0001	1	0.001
Children ever born	No child	1	0.013	1	0.0001
	1-2	4.02 (1.60, 10.06)		17.73 (4.53, 69.33)	
	3-5	4.44 (1.78, 11.06)		49.4 (10.58, 230.9)	
	> 5	4.33 (1.21, 15.58)		33.7 (4.83, 235.2)	
Current contraceptive use	Yes	1	0.026	1	0.0001
	No	1.79 (1.07, 2.98)		4.77 (2.19, 10,42)	
Number of sanitary pad usage per day	1-2	1	0.0001	1	0.004
	≥ 3	3.47 (2.04, 5.91)		3.03 (1.43, 6.41)	
BMI	< 18.5	6.49 (3.45, 12.16)		4.07 (1.69, 9.84)	
	≥ 18.5	1	0.0001	1	0.002

AOR = Adjusted Odds Ratio, COR = Crude Odds Ratio, C.I = Confidence Interval, * = History of bleeding other than menstruation and delivery

CHAPTER SIX: DISCUSSION

6.1 Discussion

Despite the fact that anemia is preventable and easily treatable, still continues to be a common cause of mortality and morbidity [16]. Anemia in women of childbearing age is a growing public health problem that justifies the implementation of interventions for its prevention and control [19]. Different strategies and intervention mechanisms for treatment and control of underline causes need to know area of focus with magnitude and clear association of associated factors. But data on relative contributions of causal factors are limited on non-pregnant women of childbearing age which makes it difficult to effectively address the problem in developing countries [6]. In addition, many reports show a variation in prevalence between different continents, countries and regions according to the burden of risk factors associated with anemia on this group of people. This indicates there should have representative, updated and group targeted data at which anemia treatment and control intervention to be focus. Therefore, current study tried to assess prevalence and associated risk factors of anemia among non-pregnant women of childbearing age, in Jimma town, south west Ethiopia.

Prevalence of anemia in this study with reference to WHO cutoff points [6] indicates mild public heath significance. A recent (2011) EDHS measured HGB levels to identify anemia [12] reported a comparable prevalence of 17%.

The prevalence of anemia in this study was found to be lower than WHO estimates [7]. This might have happened due to the fact that the model used to estimate prevalence of anemia was overestimated anemia prevalence as it was done based on nationally non representative data or survey in some countries including Ethiopia and these estimates were based on a number of assumptions like all surveys were treated equally, although in fact their quality varied greatly.

This figure is also lower than the report what has been documented by Jemal H. et al in 2010 (29.8%) [13]. This might be due to environmental and study population difference as reports indicated here were focused on both urban and rural study population. On the other hand, current

study was done using urban population and at the same time Ethiopian policy of urban health extension program might have its own contribution to be so.

Regarding to severity, 69 (15.6%) were mildly anemic and 2 (0.5%) were moderately anemic which indicates no severe anemia was identified. The result in this study was much lower than a report on anemia among non-pregnant women in Bangladesh by Ziauddin H. et al in 2001 [24] reported as prevalence of mild, moderate and severe anemia being 52%, 20% and 1%, respectively. It might be due to the same reason, environmental and study population difference, contributing low anemia prevalence compared to other reports described above as it was done 12 randomly selected villages which were representative of typical rural Bangladesh while current study was done in urban population.

As far as morphological types of anemia are concerned, 14.3% and 1.8% were normocytic normochromic and microcytic hypochromic anemia respectively. This might be due to most determinant factors identified in this study in one way or another related with increase blood loss and/or red cell destruction resulting normocytic normochromic anemia.

Non-pregnant women of childbearing age of 25 - 35 years old were 6.53 times more likely to be anemic compared to age group of 36 – 49 years old (AOR = 6.53, 95% C.I: 1.82 – 23.39; P = 0.016). This might be due to the fact that this age category is in fertility intensive women's life in Ethiopia. Similar studies on prevalence of anemia among clients of family planning clinics in Egypt by Ezzeldin O. et al in 1999 [31] reported as this age group of non-pregnant women of childbearing age were relatively more affected than other age groups. But a reports on Prevalence of anemia in women of reproductive age in Meghalaya by Sanku D. et al in 2010 [23] and on prevalence of anemia and its determinant factors among non-pregnant and pregnant women in India by Bharati P. et al in 2008 [22] indicated non-pregnant women aged less than 25 years were the most affected by anemia compared to the other age groups. It might be due to the fact that in India half of child bearing age women had a birth before they were 20 years old, and more than one in four before they were 18 years old, early childbearing is most common [37] and these two reports were done in India.

Being from lower economic level and poor educational status were identified as predisposing factors to anemia. Illiterate non-pregnant women of childbearing age were 2.16 times (AOR = 2.16, 95% C.I: 1.67 - 5.18; P= 0.005) more likely to be more anemic compared with the same group of people educated secondary school and above. Non-pregnant women of childbearing age who had < 500 Ethiopian birr household income per month were 18.84 times (AOR = 18.84, 95% C.I: 6.47 – 54.91; P < 0.0001) and who had 500 – 1,000 were 5.9 times (AOR = 5.9, 95% C.I: 2.31 - 15.09; P < 0001) more likely to be anemic compared to women who had > 1,000 Ethiopian birr household income per month. This indicates, empowering this group of women in terms of education and economic status would have positive contributions to prevent and control anemia. This was supported by similar studies in Ethiopia by Samson G. et al in 2011 [11] and in Meghalaya by Sanku D. et al in 2010 [23] which were reported such association between economic level and educational status with anemia.

Non-pregnant women of childbearing age who have not heard about anemia were 3.33 times (AOR = 3.33, 95% C.I: 1.5 - 7.42; P = 0.003) more likely to be anemic compared to women who have heard about anemia in one way or the other. This might be directly related with educational status so that empowering women on their educational status or educating them about anemia would have positive contribution to anemia prevention.

Compared to negative for IPI, current presence of parasitic infection (AOR = 3.34 95% C.I: 1.66 - 6.73; P = 0.001) was found as 3.05 times more likely to be a risk for anemia. This might be due to the fact that pathophysiologies of most identified intestinal parasites have their own contribution on blood loss and/or red cell distraction.

The effect of non-pregnant childbearing age women's reproductive role in causing anemia is clearly indicated in this study. The risk of anemia was associated with increasing parity indicated as women who have 1 – 2 children ever born (AOR = 17.73, 95% C.I: 4.53 – 69.33) 3 - 5 children ever born (AOR = 49.4, 95% C.I: 10.58 – 230.9) and >5 children ever born (AOR = 33.7, 95% C.I: 4.83 -235.2) were more affected compared with women who have no child (P = 0.0001). This might be due to the fact that there have been blood losses through each previous delivery. Report in Ethiopia by Samson G. et al in 2011 [11] and report in Meghalaya (2010)

regarding to anemia in relation to parity reported as, 1- 2 (47.2%) 3 - 4 (48%) and 5 or above (51.3%) [23] indicated increasing number of parity was a key factor predisposing women to anemia which supports this study.

The positive contribution of contraceptive use also provides supportive evidence in this regard. Those not using contraceptive (AOR = 4.77, 95% C.I: 2.19 -10.42; P = 0.0001) were more likely to develop anemia than current contraceptive users. This might be due to the fact that 96.6% of family planning users were using hormonal contraceptive methods in this study and utilization of hormonal contraceptives contributes crucially in reducing the burden of anemia as it might have its own contribution on menorrhagia, increase birth spacing between their children and reduce parity. This finding was supported by reports on prevalence & risk factors of anemia among women of reproductive age in Bursa, Turkey by Pala K. et al in 2008 [17], in Egypt by Ezzeldin O. et al in 1999 [31] in Dar-es-Salaam, Tanzania by Massawe S. et al in 2002 [27] and in Ethiopia by Samson G. et al in 2011 [11].

According to findings of this study, usage of more than 2 sanitary pads in a day during menstruation (AOR = 3.03 95% C.I: 1.43 - 6.41; P = 0.004) was found risk factor to anemia. This is also supported by a report in Bursa, Turkey by Pala K. et al in 2008 [17]. History of bleeding other than delivery and menstruation (AOR = 3.28, 95% C.I: 1.24 - 8.68; P = 0.017) was also identified as risk of anemia compared to respective category. These might be due to the fact that increasing number of sanitary pad usage per day reflects the amount of blood flow increase or it was an indication of heavy menstrual period. This might result in relatively high blood loss, predisposing factor for anemia and history of bleeding other than delivery and menstruation might have a consequence in additional blood loss among non-pregnant women of childbearing age.

Undernourished non-pregnant women childbearing age who had low BMI, <18.5 (AOR = 4.07, 95% C.I: 1.69 – 9.84; P = 0.002) were 4.07 times more likely to have anemia compared to those who have BMI ≥ 18.5. This might be due to the fact as anemia is one of the most common nutritional deficiency disorder observed globally and underlying cause of anemia includes nutritional deficiencies. Reports done in Dar-es-Salaam, Tanzania by Massawe S. et al in 2002

[27], India by Bentley M. et al in 2003 [30] and by Bharati P. et al in 2008 [22] and in Ethiopia by Samson G. et al in 2011 [11] were also reported the same relation as anemia was decreased significantly with increasing BMI.

6.2 Strength and weakness of the study

6.2.1 Strength of the study

- It was designed on community based study to identify determinant factors of anemia targeting on specific group of people called non-pregnant women of childbearing age aimed on providing an input for early prevention and control of anemia and it was done on the bases of primary data

6.2.2 Weakness of the study

- The etiology of anemia were not assessed
- Being cross sectional study design

CHAPTER SEVEN: CONCLUSIONS AND RECOMENDATIONS

7.1 Conclusions

In the present study, the prevalence of anemia was 16.1% with mean HGB concentration of 12.96 (± 1.04) of which 15.6% and 0.5 % had mild and moderate anemia respectively. Regarding to morphological types of anemia, 14.3% and 1.8% had normocytic normochromic and microcytic hypochromic respectively. According to WHO cut off value the overall prevalence of anemia in this study indicated mild public health significance.

Being younger age groups, lower educational status and economic level, history of bleeding other than menstruation and delivery, high parity or increasing number of children ever born and presence of current IPIs were identified as determinants of anemia. Number of sanitary pad usage ≥ 3 per day and under nourished (low BMI), were positively associated with anemia. Whereas, current contraceptive usage and knowledge about anemia were identified that can reduce risk of anemia.

7.2 Recommendations

Finding of this study still implies that more coordinated efforts should be paid to control important predisposing factors to anemia including IPI, chronic illness and accidents relating to blood loss.

- Family planning, economic and educational empowerment of women has affirmative inputs in combating anemia. A combination of family planning, nutrition, educational empowerment strategies should be instated through primary health care as it will have a contribution on prevention of anemia in non-pregnant women of childbearing age.

- Knowledge about anemia has a negative relation with anemia in this study. So that health professionals in every step of health institution should have health education program on anemia and its determinant factors identified.

- Peoples should participate on public health education program on anemia and its risk factors promoting early diagnosis and treatment of anemia.

- Although predisposing factors for anemia were documented so far, large scale studies should be done to assess additional associated risk factors in non-pregnant women of childbearing age.

REFERENCES

1. World Health Organization. The World Health Report 2002: Reducing risks, promoting healthy life. Geneva, WHO. 2002.

2. Lewis S. Mitchel, Bain J. Barbara, Betes I. Dacie and Lewis Practical Hematology, 10th ed. 2006.

3. Hoffbrand A. Victor, Daniel C., Edward GD. Tuddenham, Anthony R. Green, Postgraduate Hematology, 6th ed. 2011.

4. Iron deficiency anemia: assessment, prevention, and control. A guide for programme managers. Geneva, WHO. 2001

5. Ansari T, Ali L, Aziz T, Ara J, Liaquat N, Tahir H. Nutritional iron deficiency in women of child bearing ages--what to do? J Ayub Med Coll Abbottabad. 2009; 21(3): 17-20.

6. McLean E, Cogswell M, Egli I, Wojdyla D, De Benoist B. Worldwide prevalence of anemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutrition. 2008 Apr; 12(4): 444–54.

7. World Health Organization, Centers for Disease Control and Prevention. Worldwide Prevalence of Anemia 1993-2005: WHO Global Database on Anemia. 2008.

8. Alarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anemia in low-income and middle-income countries. The Lancet. 2011 Dec; 378(9809): 2123–35.

9. Jose O. Mora, Erick B., Chessa L., and Ruben G. Anemia in Latin America and the Caribbean, situation analysis, trends, and implications for public health programming. Pan American Health Organization, 2010.

10. Melaku U, Jemal H, Tsegaye D, Girma A, Gonfa A. Iron deficiency anemia among women of reproductive age in nine administrative regions of Ethiopia: Ethiop.J.Health Dev. 2008; 22(3): 252-258.

11. Samson G, Fikre E. Correlates of anemia among women of reproductive age in Ethiopia: evidence from Ethiopian DHS 2005. Ethiop J Health Dev 2011; 25 (1): 22-30.

12. Ethiopia Demographic and Health Survey 2011; Preliminary report, Central Statistical Agency, Addis Abeba, Ethiopia: ICF Macro Calverton, Maryland, U.S.A. Sep. 2011.

13. Jemal H. Prevalence of anemia, deficiencies of iron and folic acid and their determinants in Ethiopian women: J Health Popul Nutr. 2010 August; 28(4): 359–368.

14. Jemal H, Pobocik RS. Iron deficiency anemia is not a rare problem among women of reproductive ages in Ethiopia: a community based cross sectional study. BMC Blood Disord. 2009 Sep 7; 9: 7.

15. Nutrition Landscape Information System (NLIS) country profile indicators interpretation guide, WHO. 2010

16. Tolentino K, Friedman JF. An update on anemia in less developed countries. Am J Trop Med Hygiene. 2007; 77(1): 44-51.

17. Pala K, Dundar N. Prevalence & risk factors of anemia among women of reproductive age in Bursa, Turkey. Indian J. Med. Res. 2008 Sep; 128(3): 282–6.

18. Zewditu G, Kelbessa U, Timotewos G, Ayele N. Review of the status of malnutrition and trends in Ethiopia. Ethiop. J. Health Dev. 2001; 15(2): 55-74.

19. Shamah-Levy T, Villalpando S, Rivera JA, Mejía-Rodríguez F, Camacho-Cisneros M, Monterrubio EA. Anemia in Mexican women: a public health problem. Salud Publica Mex. 2003;45 Supp l4: S499-507

20. Ayoya MA, Bendech MA, Zagré NM, Tchibindat F. Maternal anemia in west and central Africa: time for urgent action. Public Health Nutrition. 2011 Oct 6; 15(05): 916–27.

21. Ribot B, Aranda N, Viteri F, Hernandez-Martinez C, Canals J, Arija V. Depleted iron stores without anemia early in pregnancy carries increased risk of lower birth weight even when supplemented daily with moderate iron. Human Reproduction. 2012 Feb 21; 27(5): 1260–6.

22. Bharati P, Som S, Chakrabarty S, Bharati S, Pal M. Prevalence of anemia and its determinants among non pregnant and pregnant women in India. Asia Pac J Public Health. 2008; 20(4): 347–59.

23. Sanku D, Sankar G, Madhuchhanda G. Prevalence of anemia in women of reproductive age in Meghalaya: a logistic regression analysis, Turk J Med Sci. 2010; 40 (5): 783-789

24. Ziauddin Hyder S, Persson Lk, Chowdhury A, Ekström EC. Anemia among non-pregnant women in rural Bangladesh. Public Health Nutrition. 2001 Feb; 4(1): 79–83.

25. Trinh LT, Dibley M. Anemia in pregnant, postpartum and non pregnant women in Lak district, Daklak province of Vietnam. Asia Pac J Clin Nutr. 2007; 16(2): 310-5.

26. Ngnie-Teta I, Kuate-Defo B, Receveur O. Multilevel modelling of sociodemographic predictors of various levels of anaemia among women in Mali. Public Health Nutrition. 2008 Dec 24; 12(09): 1462.

27. Massawe SN, Urassa EN, Nyström L, Lindmark G. Anemia in women of reproductive age in Dar-es-Salaam, Tanzania. East Afr Med J 2002; 79: 461 -6.

28. Stoltzfus RJ, Dreyfuss ML. Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia. Geneva: International Nutritional Anemia Consultative Group/UNICEF/WHO. 1998.

29. Akhwale WS, Lum JK, Kaneko A, Eto H, Obonyo C, Björkman A, et al. Anemia and malaria at different altitudes in the western highlands of Kenya: Acta Trop. 2004 Jul; 91(2):167-75.

30. Bentley ME, Griffiths PL. The burden of anemia among women in India. Eur J Clin Nutr. 2003 Jan; 57(1):52–60.

31. Hassan EO, el-Hussinie M, el-Nahal N. The prevalence of anemia among clients of family planning clinics in Egypt. Contraception. 1999 Aug; 60(2):93–9.

32. Brabin L, Nicholas S, Gogate A, Karende A. High prevalence of anemia among women in Mumbai, India. Food Nutr Bull 1998; 19:205–9.

33. Federal Democratic Republic of Ethiopia, Ministry of Health; Health Sector Development Programme IV, 2010/11 – 2014/15: version 19; October 2010

34. Central Statistical Agency, Federal Democratic Republic of Ethiopia: The 2007 population and housing census of Ethiopia, result for Oromia region. Addis Ababa, Ethiopia: Central Statistical Agency; 2010.

35. Lynch EC. Peripheral Blood Smear, Clinical Methods: The History, Physical, and Laboratory Examinations [Internet]. 3rd ed. Boston: Butterworths; 1990 [cited 2013 Jun 25]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK263/

36. Houwen B. Blood film preparation and staining procedures. Clin. Lab Med. 2002 Mar; 22(1): 1-14.

37. International Institute for Population Sciences (IIPS) and Macro International, National Family Health Survey (NFHS-3), 2005–06: India: Mumbai: IIPS. 2007.

38. Monica Cheesbrough. District Laboratory Practice in Tropical Countries, Part 1. 2nd ed. 2005.

ANNEXS

Annex I: Urine HCG test principle, procedure and interpretation

ADVANCED QUALITY™ ONE STEP Pregnancy (hCG) strip test (InTeC®) is a rapid chromatographic sandwich immunoassay for the qualitative detection of human chorionic gonadotropin (HCG) in urine/serum/plasma to aid in the early detection of pregnancy. The test utilizes a combination of antibodies including a monoclonal HCG antibody to selectively detect elevated levels of HCG. The use of specific antibody reagents ensures a highly sensitive and specific test. The test has a sensitivity of 25 mIU/ ml HCG for urine, which is sufficient to detect pregnancy the first day of the missed period. The test is specific for HCG and does not cross react with related glycoprotein hormones.

The assay is conducted by immersing the test strip in a urine sample and observing the formation of colored lines. Sample reacts with the specific anti-HCG-colloidal gold conjugated monoclonal antibody on the test membrane. This mixture moves along the membrane, by capillary action, and reacts with a specific anti-HCG in the test region. If HCG is present in the sample, the result is the formation of a colored band in the test region. If there is no HCG in the sample, the area will remain white. The sample continues to flow to the control region and forms a pink to purple color, indicating the test is working and the result is valid.

Test procedure

1. Urine has been collected in a clean, dry container. Note: First morning urine usually contains the highest concentration of HCG and is therefore the best sample when performing the urine test. However, randomly collected urine specimens may be used.
2. To begin testing, the sealed pouch has been opened by tearing along the notch.
3. Holding the strip vertically, carefully dip it into the specimen the arrow end pointing towards the urine. Did not immerse past the MAX Line (Marker Line).
4. The strip has been held in the sample until a reddish color appears at the lower edge of the test membrane (approximately 10 seconds).
5. The strip has been withdraw and placed it face up on a clean, dry surface.

6. The result was read d between 3 and 10 minutes after adding the sample. It is important that the background is clear before the result is read. Did not read results after 10 minutes

Interpretation of results

Negative: Only one color band appears on the control region. No apparent band on the test region. This indicates that no pregnancy has been detected.

Positive: Distinct color bands appear on the control and test regions. Presence of both test line and control line indicate that you are pregnant. The color intensity of the test bands may vary since different stages of pregnancy have different concentrations of HCG hormone.

Note: A positive test line appears directly below the control line on the same test surface or 'result window' area. Any line or accumulation of color/dye that appears at the juncture between test components should not be mistaken for "test line" (this is only the source of the test reagent & dye).

Invalid: No visible control band at all. The control band not appears if an insufficient volume of specimen is added into the test kit. Proper procedures may not have been followed in performing the test. Repeat with a new test kit. Please consult above instructions and follow precisely.

Storage and stability

The test kits can be stored at room temperature (2 - 30°C) in the sealed pouch to the date of expiration. The test kits should be kept away from direct sunlight, moisture and heat. Do not use test kits beyond expiration date. The test device should not be reused.

Annex II: Procedure for weight and height measurement

Weight measurement procedure

1. Study participants were asked to remove their heavy outer garments; jacket, coat and shoes
2. They have been asked to empty their pockets of trousers or skirt.
3. The participant stands in the centre of the platform, weight distributed evenly to both feet.
4. The weight has been read exactly with the arrow was aligned
5. The weight was recorded to the resolution of the scale, the nearest 0.1 kg

Height measurement procedure

1. Participants were asked to remove their shoes, heavy outer garments, and hair ornaments
2. The participants were asked to stand with their back to the height rule. The back of the head, back, buttocks, and heels should be touching the upright, feet together. The participant were asked to look straight
3. The head, sliding part of the measuring rod, was lowered so that the hair, if present, was pressed flat
4. Height was recorded to the resolution of the height rule, the nearest 0.1 centimeter

Annex III. Procedure for vacutainer venous blood collection and analysis

The Vacutainer system consists of a double-pointed needle, a plastic holder or adapter, and EDTA containing vacuum tubes with rubber stoppers. The blood goes from the subject directly into the test tube. Vein is punctured with a sterile needle attached to an aspirating device. This allows the drawing of venous blood with the least amount of patient discomfort and trauma.

Procedure:

1. the necessary materials and equipments have been assemble
2. Thread the short end of the double-pointed needle into the holder and push the tube forward until the top of the stopper meets the guide mark on the holder

   The point of the needle was embedded in the stopper without puncturing it and loosing the vacuum in the tube.
3. Identify the right subject and allow her to sit comfortably on an armchair stretching her arm and reassure.
4. The tourniquet has been applied
5. The arm has been prepared by swabbing the anticubital fossa with cotton moistened with 70% alcohol. Clean area in concentric circles starting at site and ending outside of site. Did not re-touch the cleaned area and let to air dry
6. the back of the patient's arm has been grasped at the elbow and anchor the selected vein by drawing the skin slightly taut over the vein
7. Needle has been inserted the into the vein; the index finger was placed along side of the hub of the needle with the bevel facing up and the needle has been pointed in the same direction as the vein
8. Then the point of the needle was advanced 0.5-1.0cm into the subcutaneous tissue (at an angle of 45^0) and is pushed forward at a lesser angle to pierce the vein wall

9. When the needle was properly in the vein, the vacuum tube was pushed into the needle holder all the way so that the blood flows into the tube under vacuum

10. Tourniquet has been release at the moment blood starts entering the vacuum tube

11. After drawing the required blood sample, a ball of cotton has been applied to the puncture site and gently withdraw the needle

12. Participant has been instructed to press on the cotton

13. The tube has been remove from the vacutainer holder and gently invert the tube 8-10 times

14. Tube has been labeled with subject ID before she leaves the collection area

15. Vein puncture site has been re-inspect to ascertain that the bleeding has stopped and do not let to go until the bleeding stops. If bleeding did not stopped band aid has been applied.

Procedure for blood film preparation

Thin blood film:

A thin blood film is a drop of blood that has been systematically smeared on a slide and which has more or less a monolayer of cells. It is used to the investigation and evaluation of anemia, and other conditions which produce changes in the appearance of blood cells and differential white cell count.

Procedure;

1. The necessary materials have been assembled; two glass slide, dropper, glove, well mixed EDTA anticoagulated blood

Note: It is essential to ensure slides are washed, free from traces of detergent and the surface of the slide is completely clean and not greasy.

2. A small drop of well mixed EDTA blood has been place, 1.0 cm from the end of the glass slide using blood dropping device.

3. The spreading slide was placed in front of the drop of blood at an angle of about 30^{o}-40^{o} to the slide and then is moved back to make contact with the drop

4. Spread with a smooth steady motion so that a thin film of blood was spread over the slide

5. The smear has been allowed to air-dry

    - **Do not blow** on the smears as this can disrupt cellular morphology and cause the formation of unwanted artifacts, like target cells

6. Labeled with participants unique ID or reference number on the head of the film using a lead pencil

Thick blood film

Thick blood smears are widely used in the diagnosis of hemoparasites particularly malaria. It gives a higher percentage of positive diagnosis in much less time since it uses more blood and thicker compared to thin blood film.

Procedure:

1. The necessary materials has been assembled; microscopic slide, dropper, glove, applicator stick, well mixed EDTA anticoagulated blood
2. a small drop of blood has been placed on a clean slide
3. Spread it with an applicator stick or the corner of another slide.
4. Label with participants unique ID or reference number on the frosted end of the slide using a lead pencil

The prepared smear corresponds to a circle of approximately 2cm diameter and small prints are just visible through the blood smear.

Procedure for Wright and Giemsa stains

Both stains are polychromed and are Romanowsky Stains. Acidic dye, eosin unite with the basic components of the cell, cytoplasm, and hence the cytoplasm is eosinophilic. Conversely, basic stains, methylene blue are attracted to and combine with the acidic parts of the cell (nucleic acid and nucleoproteins of the nucleus) and hence these structures are called basophilic. Other structures stained by combination of the two are neutrophilic.

Wright stain procedure

1. Air-dried smear film side has been placed up on a staining rack (two parallel glass rods kept 5cm apart).
2. Cover the smear with undiluted stain and leave for 1 minute
3. An equal volume of pH 6.8-buffered water (i.e., the same number of drops as the stain) has been added
4. It has been mixed by blowing until a metallic sheen appears and allowed to act for 3-5 minutes
5. Stain has been washed off with running tap water/wash bottle
6. The back of the slide has been wiped cleaned and stood it in a draining rack for the smear to dry (head part down).
7. It has been examined for red cell morphology. oil immersion has been used for 100X objective.

Giemsa stain

1. 10% Giemsa working solution of the required volume has been prepared.
2. Air-dried slide has been placed on staining rack (two parallel glass rods kept 5cm apart).
3. It has been covered with a 1:10 diluted Giemsa working solution and let for 10 min.
4. Stain from the slide has been washed gently using clean water (not necessarily distilled water or buffered water)
5. The back of the slide has been wiped cleaned and stood it in a draining rack for the smear to dry (head part down).
6. It has been examined for red cell morphology. oil immersion has been used for 100X objective.

Peripheral blood smear examination

Microscopic examination of the peripheral blood is used to supplement the information provided by automated hematology analyzers. Hematology analyzers provide accurate quantitative information about blood cells and can even identify abnormal cells. However, the precise classification of abnormal cells requires a trained microscopist, a well-made peripheral blood smear, and a light microscope with good optical characteristics.

Peripheral smear examination requires a systematic approach in order to gather all possible information. In addition, all specimens must be evaluated in the same manner, to assure that consistent information is obtained. The following approaches is recommended and applied:

- An examination at low power (10X ocular, 10 x objectives) is first performed to evaluate the quality of the smear to detect rouleaux formation, platelet clumps, and leukocyte clumps and other abnormalities visible at low magnification. An optimal area for evaluation at higher magnification is also chosen. This should be an intact portion of the smear free of preparation artifact where the red blood cells are separated by 1/3 to 1/2 of a cell diameter. The red blood cells should stain a pink color, while neutrophils show "crisp" features, with deep blue-purple nuclear material and pinkish to violet cytoplasmic granules. Optimal preparation and staining of the peripheral blood smear is critical for morphologic examination; an inadequate smear should not be examined.

- Following low power examination of a peripheral blood smear, 100X objective of the microscope have been selected, 1000X total magnification using a 10x ocular, and the area of morphology is examined in a consistent scanning pattern to avoid observing the same cell(s) twice and any abnormal morphology of RBCs. The size of red cell can be evaluated by using nucleus of small lymphocyte.

- If the estimate does not match the automated cell count, obtain the original blood specimen, confirm patient identity, repeat the automated analysis, and prepare a new smear [35, 36].

Scanning technique for peripheral blood morphologic evaluation was battlement method as;

- Microscopic fields were examined in a vertical direction from bottom to top or top to bottom.
- The slide has been horizontally moved for the next field
- The procedure has been repeated until 100 leukocytes have been counted.

Procedure for entering and running patient specimen in *CELL-DYN® 1800*

Prior to running samples, perform daily start-up procedures. When the **READY** message is displayed on the run screen, the instrument is ready to run specimens.

Entering specimen ID

- **Manual entry**
    1. From **RUN** screen, **[SPECIMEN TYPE**] has been press
    2. In the SPECIMEN TYPE screen, **[PATIENT SPECIMEN**] has been press
    3. The cursor has been placed in the **<NEXT ID #>** entry field. Use the alphanumeric keys on the keyboard to enter a specimen ID of up to 16 characters.

Running patient specimen

To run patient specimens, proceed as follows:

1. With the cap tightly secured on the specimen tube, it has been slowly inverted the tube 10 to 15 times.
2. Cap from the pre-mixed specimen tube has been removed.
3. The tube under the aspiration probe has been placed and raised so that the end of the probe is deeply immersed in the specimen.
4. The touch plate has been pressed to aspirate the run.
5. When the sample has been aspirated from the tube, the probe has been moved up through the wash block. Specimen tube has been removed and replaced the cap.
6. After the cycle was completed, run results were displayed on screen and the aspiration probe has been moved into position to accept a new specimen. The current run data is saved to the Data Log.
7. **[PRINT REPORT**] has been press to obtain a copy of the results.

NOTE: if a system has been idle for 15 minutes or more, a normal background should be run immediately prior to running patient specimens.

Annex IV: Procedure for stool examination

Direct wet mount microscopic examinations for detection of intestinal parasites

The direct examination of faces is essential to detect motile parasites and is usually adequate to detect some IPI.

Procedure

1. A drop of saline was placed on the microscope slides
2. With applicator stick a small amount of faeces (equivalent to the size of a match head) has been picked up and mixed with a saline drop
3. Cover slip has been put on the preparation
4. Finally the preparation was examined under 10x and 40x objective of the microscope to detect parasites from the stool

Formol-ether concentration technique for stool examination

Principle

Feces were emulsified in formol water, the suspension was strained to remove large fecal particles, diethyl ether was added, and the mixed suspension was centrifuged. Cysts, oocysts, eggs, and larvae were fixed and sedimented and the fecal debris was separated in a layer between the ether and the formol water. Fecal fat was dissolved in the ether.

Procedure

1. Using a rod or stick, an estimated 1 gm (pea-size) of faces has been emulsified in about 4 ml of 10% v/v formol water contained in a screw-cap bottle or tube
2. Further 3–4 ml of 10% v/v formol water has been added, caped and well mixed by shaking.
3. Emulsified feces has been sieved collected in a beaker
4. The suspension has been transferred to a conical (centrifuge) tube and 3–4 ml of diethyl ether has been added
5. It has been cupped and mixed for 1 minute
6. With piece of cloth, it has been wrapped around the top of the tube loosen the stopper (considerable pressure has built up inside the tube).

7. It has been centrifuged at 3000 rpm for 1 minute. After centrifuging, the parasites have been sediment to the bottom of the tube and the fecal debris have been collected in a layer between the ether and formol water
8. Using a stick or the stem of a plastic bulb pipette the layer of fecal debris has been loosen from the side of the tube and the tube has been invert to discard the ether, fecal debris, and formol water. The sediment remains.
9. The tube has been returned to its upright position and allowed to drain to the bottom. Tap the bottom of the tube to re-suspend and mixed the sediment. Transfer the sediment to a slide, and cover with a cover glass.
10. It has been examined microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective to examine small cysts and eggs.

Although the motility of Strongyloides larvae was not seen, the non-motile larvae have been easily recognized [38].

Annex V: Questionnaire in English version

Jimma University

College of Public Health and Medical Sciences

Department of Medical Laboratory Sciences and Pathology

Introduction

Good morning/afternoon, my name is ……..……..came from Jimma university and I am here with my colleagues to study about anemia. The main objective of this study is "To determine the prevalence of anemia and associated risk factors among non-pregnant childbearing age women in Jimma town, South West Ethiopia". This questionnaire is intended to assess the knowledge, practice and other potential risk factors related to anemia. If you agree, I would like to obtain 4ml of venous blood specimen and stool specimen in plastic sheet from you, which would be used only to detect the presence of anemia, hemoparasits and intestinal parasites. You will not get any risk in participating in the study rather it has a contribution in reducing the burden of anemia. If you will found to be positive for anemia, malaria and/or any intestinal parasite infections, you will have a drug prescription for treatment (free of charge for anemia).

The information in your records will be strictly confidential and your participation is completely voluntary that you can refuse to participate or withdraw yourself from the study at any time. But your refusal for participate will not result in loss of medical treatment or any other benefits if needed.

Do you understand what has been said to you? If you have questions, you have the right to get proper explanation. We will give enough time to think over before you sign on informed consent.

Are you willing to participate in the study? 1. Yes 2. No

We thank you for your participation in the study.

Part One: - Anthropometric, Socio-demographic and Socio-economic information of study participant

Ser.No	Question	Answers	Remark
100	Anthropometric measurement	Height in centimeter _____ Weight in kilogram _____	
101	Age	-----	
102	Marital status	1. Unmarried 3. Widowed 2. Married 4. Divorced	
103	Educational status	1. Illiterate 2. Read and write 3. Grade 1-4 completed 4. Grade 5-8 completed 5. Grade 9-12 completed 6. College/University completed	
104	Occupation	1. Employee 2. Housewife 3. Daily labourer 4. Student 5. Other -------	
105	Members of the household	_____	
106	Monthly household income	_____	

Part two: - questionnaire intended to assess knowledge, practice and other potential risk factors related to anemia

201	Have you ever heard about anemia?	1. Yes 2. No	
Ministration related questions			
202	Regularity of cycle:	1. Regular 2. Irregular	
203	Length of flow in each menses (days):	_____	

204	Number of sanitary pad usage (per day):	_____	
Family planning and parity related questions,			
205	Have you use any family planning method currently?	1. Yes 2. No	
206	If your response in Q 205 is yes, what type is it?	_____	
207	Parity /Children ever born	1. Yes 2. No	
208	If your response in Q 207 is yes, how many?	1. Live birth _____ 2. Stile birth _____ 3. Abortion _____	
209	Where you have got service of the last delivery?	1. Health institution, 2. Trained traditional practitioner, 3. In your house without the help of practitioners	
210	Are you on the state of breastfeeding currently?	1. Yes 2. No	
211	What is the birth spacing time/period between your last two children?	_____	
Health related questions			
212	Have you ever bled other than delivery and menstruation?	1. Yes 2. No	
213	Chronic illness (MTB)	1. Yes 2. No	

IPI = Intestinal Parasitic Infection, MTB = Mycobacterium tuberculosis, Q = Question

Annex VI: Questionnaire in Amharic version

ጅማ ዩኒቨርስቲ

የህብረተሰብ ጤና እና የህክምና ሳይንሶች ኮሌጅ

የህክምና ላብራቶሪ ሳይንስ እና ፓቶሎጂ ትምህት ክፍል

መግቢያ

እንደምን ዋሉ/አደሩ፣ ስሜ …….…….. ይባላል፡፡የመጣሁት ከጅማ ዩኒቨርሲቲ ሲሆን ከጉአደኛዬ ጋር እዚህ የተገኘነዉ ስለ ደም ማነስ ጥናት ለማድረግ ነዉ፡፡የዚህ ጥናት ዋና አላማ የደም ማነስ በጅማ ከተማ ዉስጥ በሚኖሩ ነፍሰጡር ያልሆኑ ሴቶች ላይ ያለዉን ስርጭትና የደምማነስ እንዲከሰት የሚያደርጉ ኢጋላጭ ምክንያቶችን ለማጥናት ሲሆን ይህ ቃለ መጠይቅ የተዘጋጀዉ ህብረተሰቡ ስለ ደም ማነስ ያለዉን እዉቀት አመለካከትና ትግበራ እንዲሁም ህክምና የመፈለግ ዝንባሌና ሌሎች ኢጋላጭ ምክንያቶችን ይዳስሳል፡፡ በትናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ለዚህ ጥናት ያገለግል ዘንድ ከክንድዎት ጥቂት ደምና 5ግም ያህል የሰገራ ናሙና ከርሶ እንዲወሰድ ይሆናል፡፡ናሙናዉ የሚያገለግለዉ የደም ማነስና የሆድ ትገኛ ትላትል መኖርና አለመኖሩን ለማወቅ ብቻ ነዉ፡፡ እርስዎም በዚህ ጥናት ተሳትፎ ቢያደርጉ ምንም አይነት ጉዳት የማያስከትልብዎ ሲሆን በምርምር ዉጤት ላይና በከተማ የደም ማነስ የሚያስከትለዉን ጫና ለመቀነስ ግን የጎላ አስተዋፆ አለዉ፡፡

በመጠይቁ ወቅት የሚሰጡት መልሶች እና አስተያየቶች በሙሉ በምስጢር የተጠበቁ ይሆናሉ፡፡ በዚህ ጥናት ላለመካፈልና በመሀል በማንኛዉም ጊዜ ለማቆም መብትዎ የተጠበቀ ነዉ፡፡ነገር ግን ባለምሳተፍዎ ምክንያት የህክምና ወይም ሌሎች ጥቅማጥቅም አስፈላጊ በሆነ ሰዓት ስንዲያጡ ምክንያት አይሆንም፡፡

እስካሁን የተባለዉን ተገንዝበዉታል? ጥያቄ ካለዎት ማብራሪያ ማግኘት ይችላሉ፡፡ ከመፈረምዎ በፊት እንዲያስቡበት በቂ ሰዓት እንሰጥዎታለን፡፡

በዚሁ የጥናትና ምርምር ለመሳተፍ ፍቃደኛ ኖት? 1.አዎን 2.አይደለም

በጥናትና ምርምር በመሳተፈዎ በጣም እናመሰግንዎታለን!

ክፍል 1:- በጥናቱ የሚካተቱ ሰዎች የሰዎነት ልኬት፣ ማ ህበራዊ፣ ሥነ ሕዝባዊ ና ገቢን የሚመለከቱ መረጃዎች

ተ.ቁ	ጥያቄ	መልስ	አስተያየት
100	የሰዎነት ልኬት	ቁመት በ ሴ.ሜ _____ ክብደት በ ኪ.ግ _____	
101	ዕድሜ	-----	
102	የጋብቻ ሁኔታ	1. ያገባች 3.የተፋታች 2.ያላገባች 4. የሞተባት	
103	የት/ት ደረጃ	1.ያልተማረች 2.ማንበብና መፃፍ የምትችል 3. ክፍል 1-4 ያጠናቀቀች 4. ክፍል 5-8 ያጠናቀቀች 5. ክፍል 9-12 ያጠናቀቀች 6.ኮሌጅ/ዩኒቨርስቲ ያጠናቀቀች	
104	ሥራ	1. የቅጥር ሠራተኛ 2. የቤት እማቤት 3. የቀን ሠራተኛ 4. ተማሪ 5. ሌላ ከሆነ ይግለፁ--------.	
105	የቤተሰብ ቁጥር	----------	
106	የቤተሰብ የወር ገቢ መጠን	----------	

ክፍል 2፡ ስለ ደም ማነስ እዉቀትን፣ተግባረንናሌሎችተዛማጅምክንያቶች የሚዳስሱ ጥያቄዎች

201	ስለ ደም ማነስ ከዚህ በፊት ሰምዉ ያዉቃሉ?	1. አዎ 2. ሰምቸ አላዉቅም	
የወር አበባን በተመለከተ			
202	የኡደቱ ተከታታነት	1. ተከታታይ 2. ተከታታይ አይደለም	
203	ደም የሚፈስበት ቀን ብዛት	_____	
204	በቀን የሚጠቀሙት የሞዲስ ብዛትስንት ነዉ?	_____	
ወሊድና ቤተሰብ ምታኔ የተመለከቱ ጥያቄዎች			
205	በአሁኑ ሰዓት የቤተሰብ ምጣኔ ተጠቃሚ ነዎት	አዎ 2. የለም	
206	የጥያቄ 205 አዎ ከሆነ፣ የተጠቀሙት ምድን ነዉ	……..	

207	ወልደዉ ዉቃሉ?	2. አዎ 2. የለም	
208	የጥያቄ 207 አዎ ከሆነ፣ ቁትራቸዉ ስንት ነዉ?	1. በህይወት የተወለዱ……. 2. ሞተዉ የተወለዱ …… 3. ዉርጃ …….	
209	የመጨረሻ ልጅዎን ሴወልዱ የወሊድ አገልግሎት ያገኙት የት ነዉ?	1. በጤና ድርጅት 2. ከጤና ድርጅት ዉጪ በሰለጠኑ የልምድ አዋላጆች 3. ከጤና ድርጅት ዉጪ ባልሰለጠኑ አዋላጆች	
210	በአሁኑ ሰዓት ጡት ያጠባሉ?	1. አዎ 2. የለም	
211	በመጨረሻዎቹ ሁለት ተከታታይ ልላጆችዎ መካከል ያለዉ የዕድሜ ልዩነት ስንት ነዉ?	……	
ጤንነትን የተመለከቱ ጥያቄዎች			
212	ከወሊድ ዉጭ ደም ፈሶዎት ያዉቃል?	1. አዎ 2. የለም	
213	ለረጅም ጊዜ የቆየ ህመም (ቲቢ) አለብዎ?	1. አዎ 2. የለም	

Annex VII: Questionnaire in Afan Oromo version

Jimmaa universtii

Fayyaa umaafi coolleejii sayinsistoota yalaa

Sayiinsii labiratoorii, mana yalaa fi kuta barumsaa patolloji

Seensa

Akkam oltanii bultanitu maqaan koo _____ jedhamaa. Kaniin dhufee universitti jimmaa yommuu ta'uu hirriyyaa koo wajjin asitti kan argamne qorannoo hir'ina dhiiga qoraachuuf. Hir'inaa dhiiga magaala jimmaa keessatti dubartoota ulfaa hin tanee keessa jiru tamsasaa hir'ina dhiiga akka kan uman godhuu. Sababni hir'ina dhiiga akka ummamuu kan godhuu kan nama saxiluu qorachuu yeroo ta'uu gaffiin afanii kan qopha'ee saabnii keenyaa sababa hanqinaa dhiiga akka baru beekumsaa isaa guddisuu fi akkuma kanas ilalchaa ummataa jijjirudhan akka ummatnii yalamu barbaduu fi hin barabanee sababa nama saxilu kana qorachuun ni yalaamaa qorannoo kana irraatii hirmachuuf fedhii waan qabdanu yoo ta'ee qorannoo kana akka tajajjiluuf irree keessan irraa dhiiga xinnoo girama 5 kan ta'u udaan namunaadhaaf akka fudhamu ta'a.

Namunaan Kun kan fayyaduu hir'inaa dhiigaf dhukkubaa garaa keessatti argaman kan akka rammoo fakkatan jiraachuuf akka hin jiran beeku qofaafi isiinis qorannoo kana irraatti hirmachuu rakkinaa hommaa iyyuu waan isiin irratti hin dhumne yeroo ta'uu bu'aa qorannoo fi magaala keessatti hanqini dhiiga dhiibbaa inni umuu xinneesuuf garuu hirmannaa guddaa qaba.

Gaaffii gafatamuu deebiin keenamu fi yaadi ilaalchaa namni kam iyyuu keennu hundumtu iciitidhaan waan egaamuu ta'u. Qorannoo kana irratti hirmachuu dhisuuf ykn gidduudhan qorannotti hirmachuu dhabuun mirgi keessan kan egame ta'a. Haa ta'uu malee sababa qorannoo irratti hirmachuu dhistaniif yalamuu ykn faayidaa adda addaa yeroo barbaddanu akka dhabdaniif sababa hin ta'u.

Ammaa yonaa kana olitti kan jedhamee isinii galeera? Yoo gaaffii qaabatan ibsi akka hisiniif taasifamuuf mirga qabdu. Idoo waliigaltee kana hin maallatteessini dura akka irratti yaadduuf yeroon gahaan siif kennameera.

Qoranno kana irratti hirmaachuudhaaf fedha qabdaa? 1. Eeyee 2. lakki

Hirmaanna keessanif baay'ee isin galateefanna!

Kutaa 1ffaa Safartuu qaama namaa, odeefannoo haala jereenya fi qabeenyuma namoota qorannorratti hirmaatani.

Laku	Qaafi	Deefi	Yaada
100	Safartuu qaama namaa	Ulfaatina saantimeetiraan _____ Dheerina kiiloogiramaan _____	
101	Umuri	_____	
102	Herumsa	1. Hin herumnee 3. Abba manaa dua'ee 2. Herumera 4. Hikeera	
103	Sadarka barumsa	1. Hin baranne 2. Dubbisuu fi barreesuu 3. Kutaa 1-4 fixeera 4. Kutaa 5-8 fixeera 5. Kutaa 9-12 fixeera 6. Kolleji/university fixeera	
104	Hojii	1. Hojetaa mootummaa 2. Hadha manaa 3. Hojeeta guyaa (gidoo) 4. Barataa 5. kan biroo ----------	
105	Miseensota maatii	_____	
106	Galii maatii ji'ani	----------	

Kuutaa 2ffaa: - gaaffilee waa'yee beekkumsa, shaakallii fi sababoota hir'ina dhiigaa fidaniin walqabatan

201	Waayee hir'ina dhiigaa dhageessee beektaa?	1. Eeyyeen 2. Lakki	

Gaaffii waa'ee laguutiin wal qabate			
202	Walfakkeenya ji'a ji'aan	1. Walfakkaata 2. Wal hin fakkaatu	
203	Yeroo turtii laguu (dhiiguu) (guyyaadhaan)	_____	
204	Guyyaatti paadii hammamii fayyadamtu	_____	
Gaaffilee karoora maatii fi ulfaan walqabatan			
205	Yeroo ammaa karoo maatiitti fayyadamaa jirtaa?	1. Eeyyeen 2. lakki	
206	Deebiin kee gaaffii 205 eeyyeen yoo ta'e, maal faa fayyadamta?	_____	
207	Kana dura deesssee beektaa?	1. Eeyyeen 2. lakki	
208	Deebiin kee gaaffii 207 eeyyeen yoo ta'e, meeqa?	1. Lubbuu dhan kan jiran 2. Badanii kan dhalatan 3. Kan bahee (yeroon osoo hin qahin	
209	Tajaajila dahumsaa eessatti argatta?	1. Dhaabbilee fayyaa 2. Deessistoota aadaa biratti 3. Mana keetitti 4. Kan biraa _____	
210	Yeroo ammaa harma hoosisaa jirtaa?	1. Eeyyeen 2. lakki	
211	Yeroo hammam hammamiitiin deessa?	_____	
Gaaffilee fayyaadhaan walqabatan			
212	Sababa dahumsaatiin ala sababa birootiin dhiigdee beektaa?	1. Eeyyeen2. Lakki	
213	Dhukkuba sombaa qabdaa?	1. Eeyyeen 2. Lakki	

Annex VIII: Consent form in English version

Participant Code No ----------

Participant Kebele----------

Participant Age----------

Consent Form

I have been informed about a study that plans to investigate the prevalence of anemia in Jimma town with the title of **"**Prevalence and risk factors associated with Anemia among women of childbearing age in Jimma town, south west Ethiopia" which will help in understanding of risk factors for anemia and investigating its extent to which whether or not anemia is a public health problem in the area. This study could contribute to in recommending the use of appropriate policies and control measures that can have a power to minimize the burden of the disease in the area.

For the study I have been requested to give blood and stool sample and if positive result is observed treatment will be given by using the standard drug regimen. Based on this, I have agreed to continue the examination. The investigator also informed me that all the laboratory results would be kept confidential. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperates at my willingness in the course of the conduct of the study.

Name (participant) ----------Signature ----------Date ---------

Name (investigator) ----------Signature ----------Date ----------

Thank you for your participation!

Annex IX: Consent form in Amharic version

የተሳታፊዉ ስም መለያ ቁጥር----------

የተሳታፊዉ ስም ቀበሌ----------

የተሳታፊዉ ስም እድሜ----------

የስምምነት ቅጽ :-

እኔ በጅማ ከተማ ዉስጥ ነፍሰጡር ባልሆኑ እናቶች ላይ በሚከሠተዉ የደም ማነስ ስርጭተና በሽታዉ እንዲከሰት የሚያደርጉ ኢጋላጭ ምክንያቶች በሚል ርዕስ ለማጥናት በታቀደዉ ጥናት ዉስጥ እንድሳተፍ ፍቃደኛ መሆኔንና አለመሆኔን ተጠይቄአለሁ፡፡የጥናቱ ዋና አላማዉ ይህ በሽታ በከተማችን ዉስጥ እንዲከሰት የሚያደርጉ ኢጋላጭ ምክንያቶችን ለማጥናትና በርግጥም እነዚህ ምክንያቶች ለደም ማነስ በሽታ የሚያጋልጡ ሆነዉ ከተገኙ አስፈላጊዉን ጥንቃቄ በመዉሰድ በሽታዉን ለመቀነስ የሚረዱ መሆኑ በቅድሚያ ተነግሮኛል፡፡

ለዚህ ጥናት ያገለግል ዘንድ በፈቃደኝነት ላይ የተመስተ የደምና የሰገራ ምርመራ እንዳደርግ ተጥይቄአለሁ፡፡ በምርመራዉ የደም ማነስ እና/ወይም የሆድ ጥገኛ ትላትል የተገኘ እንደሆን አስፈላጊ መድሃኒት በነጻ እንደሚሰጠኝ ተነግሮኛል፡፡ በዚህም መሰረት እኔ ምረመራውን ለማድረግ የደምና የሰገራ ናሙና ለመስጠት ተስማምቼለሁ፡፡ ማንኛውም ውጤት በሚስጢር እንደሚዝም ተነግሮኛል፡፡ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን ከመፈረሜ በፊት እንዳስብበት በቂ ጊዜ ተሰጥቶኝ የተስማማሁ መሆኔን በፌርማየ ለማረጋገጥ እወዳለሁ፡፡

የተሳታፊው ስም----------ፊርማ----------ቀን----------

የአጥኝው ስም----------ፊርማ----------ቀን----------

በጥናትና ምርምር በመሳተፈዎ በጣም እናመሰግንዎታለን!!

Annex X: Consent form in Afan Oromo version

Hirmataa Lakk. Addaa _____

Hirmataa Ganda _____

Hirmataa umuri_____

Boca waalii galte:

Ani magaala jimmaa keessatti dubarti ulfaa kan hin tanee irraati hir'ina dhiiga umamuu fi tamsasa dhukkuba akka umamuu kan godhanuu sababa nama kanaraatti saxiluu mata duree jedhuun. Qorachuuf sagantaa ba'ee keessatti hirmachuuf fedhii akkan qabuu fi akka hin qabnee gafatameera. Kayyoo guddaan qormatichaa dhukkubni Kun magaala keenya keessatti akka ummamuu sababa saxilichaa qorachuu fi dhuguman iyyuu sabaabooni kuni dhukkuuba hanqina dhiigaa waan nama saxilanu ta'ani yoo argamani of egannoo barbachiisan fudhatamee dhukkubicha hanbisanu (xiqeesuu) waan fayyadamu ta'uu isaa duran dursee natti himameera. Qorannoo kanaaf akka fayyaduu fi fedhii koo irraatii hunda'u dhaan qorannoo dhiigaa fi udaaniin akkan godhu gafatamera.

Qorannoo kana irraattis hanqina dhiigaa ykn dhukkuba garaa keessatti raamoon yoo argamee qorichi barbaachisaa ta'e bilisaan akka naaf keenamuu natti himaamera. Qorannoo kana irraatti hirmachuufi fedhii qabaadhee mallaateessuun dura akkan itti yaduu yeroo ga'aan na keename akkan waali galee mallattoo kootiin mirkanesuun naan jalaadha.

Maqaa Hirmaata _____Mallattoo _____Guyyaa_____

Maqaa Qorataa_____Mallattoo_____Guyyaa_____

Hirmaanna keessanif baay'ee isin galateefanna!

Annex XI: Laboratory results and anthropometric measurement registration form

Age_____

ID.NO _____

Kebele _____

1. **anthropometric measurement**

   Height in meter _____

   Weight in Kg _____

2. **HCG test**

   ☐ Positive for HCG

   ☐ Negative for HCG

3. **Stool examination result**

Method	**Trophozoite of:**		**Ova of:**		**Cyst of:**		**Larvae of**	
Direct wet mount	1		1		1		1	
	2		2		2		2	
	3		3		3		3	
Formol-ether concentration methode			1		1		1	
			2		2		2	
			3		3		3	

4. **CBC CELL-DYN 1800 hematology analyzer results**

Parameter and unit of measure	**RBC parameters**				**HGB parameters**		
	RBC (cells/ L)	MCV (fl)	HCT (%)	RDW%	HGB (mg/dl)	MCH (pg)	MCHC%
Result							

5. **Red cell morphology results (microscopic)**

   ☐ Normosytic normo chromic

   ☐ Micrcytic hypochromic

   ☐ Macrocytic

6. **Blood film examination**

hemoparasite	**Type and stage of parasite**		**Semi quantitative**
Plasmodium species	1		
	2		
Other hemoparasite			

Annex XII: Drug prescription format

Name of the participant _____

Age_____ Sex_____

Kebele_____

HH. No _____

ID. No _____

Diagnosis----------

Treatment prescribed----------

Name of Physician/clinician---------- (MD, HO, NUR)

Sign----------Date----------

Declaration

I, the undersigned, declare that this thesis is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. Where other peoples work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Name of the Principal investigator	**Signature**	**Date**
Yaregal asres	_____	_____
Approval of the first Advisor	**Signature**	**Date**
1. Dr. Tilahun Yemane (MD, MSc, A. professor)	_____	_____
2. Lealem Gedefaw (BSc, MSc)	_____	_____
Approval by internal examiner	**Signature**	**Date**
_____	_____	_____
Head of the Department	**Signature**	**Date**
_____	_____	_____