ANEMIA AND ASSOCIATED FACTORS AMONG PREGNANT WOMEN IN SOUTH SUDANESE REFUGEES IN PUGNIDO, GAMBELA, WESTERN ETHIOPIA

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ABSTRACT

***Background*:** Anemia is a global public health problem that mainly affects pregnant women and children. Anemia during pregnancy results in increased feto-maternal morbidity and mortality, especially in malaria endemic areas. Inadequate access to micronutrients, malaria infection and intestinal parasitic infestation in refugee environments can play a substantial role in exposing pregnant women to anemia.

***Objective*:** To assess the prevalence, severity, type and associated factors of anemia among pregnant women in South Sudanese refugees attending antenatal care Clinic at Pugnido Administration of Refugee and Returnee Affairs Health Center, Gambela, Western Ethiopia.

Methods: A facility-based cross-sectional study involving 360 pregnant women was conducted in Pugnido Administration of Refugee and Returnee Affairs Health Center from April 15 to June 30/2015. Data collection was done after obtaining informed consent. Socio-demographic, obstetric and nutritional data were collected using interviewer administered questionnaire. Mid-upper arm circumference measurement was taken. Complete blood cell counting was done using CELL-DYN 1800 (Abbott Laboratories Diagnostics Division, USA). Peripheral blood smear was examined for red blood cell morphological analysis and hemoparasite identification. Stool specimen was examined for intestinal parasites detection. Anemia during pregnancy was defined as hemoglobin concentration <11 g/dl. Statistical analyzes were done using Statistical Package for Social Sciences (SPSS) version 20.0 for windows. Association between dependent and independent variables was analyzed by logistic regression, and variables with P-value <0.05 were considered statistically significant.

***Result*:** The overall prevalence of anemia was 36.1%. Majority of the anemic pregnant women had mild form (89.2%), and the normocytic-normochromic (56.2%) type of anemia. The mean hemoglobin concentration was 11.3 ± 1.5 g/dl. Being in third trimester of pregnancy (AOR= 3.12, 95%CI:1.16-9.83), eating meat at most once a week (AOR= 2.00, 95%CI:1.11-3.58), drinking tea immediately after meal at least once a day (AOR= 3.01, 95%CI:1.74-5.22), having mid-upper arm circumference below 21 cm (AOR= 3.90, 95%CI:1.94-7.84) and intestinal parasitic infestation (AOR= 2.17, 95%CI:1.20-3.91) were significantly associated with anemia.

Conclusion: Anemia prevalence was a moderate public health problem, majority of which was mild and normocytic-normochromic type. Third trimester, intestinal parasitic infestation, low mid-upper arm circumference, less frequent eating of meat and repeated tea intake had increased the risk of anemia among pregnant women. These identified factors should be considered for prevention and control of anemia with particular attention on ensuring optimal micronutrient status among the pregnant women in the study area.

***Key words*:** Anemia, Associated factors, Pregnant women, South Sudanese Refugees, Pugnido, Western Ethiopia

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ABBREVIATIONS AND ACRONYMS

ANC: Antenatal Care

AOR: Adjusted Odds Ratio

ARRA: Administration for Refugee and Returnee Affairs

BSc.: Bachelor of Science

CBC: Complete Blood Count

CI: Confidence Interval

COR: Crude Odds Ratio

CSF: Cerebrospinal Fluid

EDHS: Ethiopian Demographic Health Survey

EDTA: Ethylenediaminetetraacetic Acid

GDP: Gross Domestic Product

Hb: Hemoglobin

HCT: Hematocrit

LBW: Low Birth Weight

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

mg/dl: Milligram per Deciliter

ml: Millilitre

µl: Microliter

MSc.: Master of Science

MUAC: Mid-upper Arm Circumference

NGOs: Non-Governmental Organizations

PLT: Platelet

P. *falciparum:* Plasmodium *falciparum*

P. *malariae*: Plasmodium *malariae*

P. *ovale:* Plasmodium *ovale*

P. *vivax:* Plasmodium *vivax*

pH: Potential of Hydrogen

QC: Quality Control

RBC: Red Blood Cells

SOP: Standard Operating Procedure

UNHCR: United Nations High Commissioner for Refugees

UNICEF: United Nations International Children's Emergency Fund

WBC: White blood cell

WHO: World Health Organization

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

CHAPTER ONE: INTRODUCTION

1.1. Background

Anemia is a condition characterized by decrease in the number of red blood cells (RBCs) or their oxygen-carrying capacity to meet physiological needs of the body. It varies by age, sex, altitude, smoking and pregnancy condition(1,2). Anemia is considered to exist when the hemoglobin (Hb) level in the blood is below 13 g/dl, 12 g/dl and 11 g/dl in adult males, adult females and children (6 to 59 months), respectively considering at sea level(3,4). Anemia is mainly caused by nutritional deficiency particularly iron deficiency, chronic infections, acute and chronic blood loss, and hereditary Hb defects(1).

The laboratory diagnosis of anemia can be done by analyzing any of the three concentration measurements: Hb level (g/dl), hematocrit (%), RBC number (10^{12}/l). Previously, these parameters were measured using manual physical and chemical techniques, but currently these tests are done by electronic cell counters and Hb analyzers(2). The modern analyzers directly measure the RBCs number, the Hb concentration, and mean corpuscular volume (MCV) that indicates an average volume of a red cell. These modern analyzers use the directly measured values to calculate the hematocrit, the mean corpuscular Hb (MCH), and the mean corpuscular Hb concentration (MCHC). These electronic counters also generate the red cell distribution width (RDW), which is a quantitative measure of the variation in red cell size(1,2).

Anemia can be classified into three types based on morphologies of the RBCs. These are microcytic-hypochromic (RBCs with low MCV and MCHC), normocytic-normochromic (RBCs with normal MCV and MCHC) and macrocytic-normochromic (RBCS with high MCV, but normal MCHC)(1,2).

Anemia during pregnancy is defined as Hb level less than 11 g/dl(1,5). The severity of anemia during pregnancy ranges from mild to severe based on the specified cut-off points for Hb concentration designated by World Health Organization (WHO)(4,6,7). According to the WHO, the anemia in a pregnant woman is categorized as severe, moderate and mild, if the blood Hb concentration is <7 g/dl, from 7 to 9.9 g/dl and from 10.0 to 10.9 g/dl, respectively(4).

Pregnancy involves many changes in maternal physiology including alterations in hematological parameters. These physiological changes include expansion of maternal red cell mass by about 20-50% and plasma volume by 40-60%(8,9). This disproportional increase in red cell mass and plasma volume during pregnancy causes hemodilution that can be expressed by fall in hematocrit value. During this

period, as the oxygen delivering capacity of blood is not interrupted the condition is referred to as physiological anemia, provided above the specific cut-off point(8,10).

Anemia in pregnancy can be due to physiological or pathological causes. In pathologic anemia of pregnancy, the oxygen-carrying capacity of the blood is deficient due to problem in erythrocyte production or their excessive loss through destruction or bleeding(10). Problem in production of erythrocytes may result from nutritional deficiency of iron, folic acid, vitamin B-12 or from hereditary Hb defects such as sickle cell(6,10). Iron deficiency is the predominant cause of pathological anemia during pregnancy, which is characterized by meeting fetal requirements for iron despite maternal deficiency(5).

During pregnancy, 3.5-4.0 mg of iron is daily required making a total of about 1000 mg iron requirement for the increased red cell mass synthesis, fetal development and replenishing basal loss during the whole length of pregnancy(9). These requirements exceed the usual storage iron (~300 mg) of most young women, and it is very difficult to be met by the diet only(5). Thus, there is a high risk of developing iron deficiency anemia that can be exacerbated if pre-conception iron stores are reduced(10). Iron deficiency is common among pregnant women, particularly those mainly eat foods from plant sources than meat(11).

Excessive loss of erythrocytes through destruction can be associated to parasitic infection. Hookworm and Plasmodium *falciparum* (P. *falciparum*) infections are among the major parasitic infections leading to anemia in developing world(2,12). Malaria is probably the major cause of anemia in areas where it has stable transmission. In these areas, malaria during pregnancy can lead to severe form of anemia that is mostly associated with bad consequences(13).

The socio-demographic environment where pregnant women exist has an eminent effect on their wellbeing. Conflict and drought that oblige for migration expose pregnant women for more troubles. The nature of the lifestyle in refugee settings have a major impact on women`s health conditions. Especially, pregnant women in refugee camps may be exposed to extreme poverty that can be expressed by nutritional deficiency, lack of access to safe water and education. These factors pose possible threats for physical and psychological health of these women; anemia is one of the leading consequences of this misery that could be worsened by inadequately addressing situation specific healthcare needs in the refugee camps(11,14).

Anemia during pregnancy results in plenty of detrimental consequences on the mother and the infant. The main consequences are higher risk of maternal and infant death, and increased risk for delivery of pre-term and low birth weight (LBW) infant(5,13). Moreover, the negative consequences of anemia on infants

does not end at birth, rather continuous in the subsequent stages and affect their cognitive and physical development(6,15).

The management and prevention of anemia in pregnant women is essential for the wellbeing of both the mother and the fetus. Anemia management depends on careful assessment of the underlying cause and taking corrective actions with respect to the specific causes. Once a pregnant women has become iron deficient, it is difficult to ensure repletion through diet alone; and hence, need for supplementation(16). Iron supplementation during pregnancy prevents anemia by replenishing maternal iron deficiency. Currently, WHO recommends 60 mg iron and 400 µg folic acid supplementation per day for pregnant women for six months where anemia is widespread(11). For making the supplementation process easy these hematinics are combined in one tablet to effectively deal with the problem(7).

The goal of anemia management during pregnancy is to maintain health by resolving it within shortest possible time, improve quality of life and assure survival of the mother and her fetus. This goal is better achieved when supported by available specific local data on prevalence and associated factors of anemia(6).

1.2. Statement of the Problem

If anemia is considered as a disease, undoubtfully, it would be regarded as the commonest disease in the world(10). Anemia is reported as one of the top ten most important contributors of global morbidity and mortality by WHO. About a third of the world population has Hb level that fall in the WHO criteria for diagnosis of anemia. The majority of these cases live in Sub-Saharan Africa and Southeast Asia(6).

World Health Organization classified the burden of anemia as a problem of public health based on its prevalence among the population in a particular area. According to the cut-off value set by WHO, anemia is not considered as a public health problem if its prevalence is 4.9% or less, but it is considered as mild, moderate and severe public health problem if its prevalence is 5.0%- 19.9%, 20.0%- 39.9%, and 40.0% or more, respectively(4,15).

Although both males and females of all ages are affected by anemia, the main vulnerable groups are pregnant women and young children due to increased iron requirement(17,18). Globally, anemia in women is a persistent problem affecting above 500 million, especially in Asia and Africa(17). Non-pregnant women of child-bearing age are among the heavily affected groups: 468 million (30.2%) in the world are anemic(15).

Anemia is terribly prevalent among pregnant women as 56.4 million (41.8%) of them are suffering from it(15). Although the burden of anemia among pregnant women in developing world is higher, it is a moderate public health problem in over 80% of world countries(15,17). Based on WHO global database on anemia, there is no country in the world where maternal anemia is not at least a mild public health problem. Therefore, anemia is a public health problem of pregnant women in all WHO member states(15).

In Africa, the prevalence of anemia in pregnancy is a serious continental issue where about 57.1% or 17.2 million cases reside(15). In Sub-Saharan Africa, which is one of the malaria-endemic parts in the world, P. *falciparum* is the major contributor to anemia in pregnancy. In this area, around 400,000 pregnant women develop moderate or severe anemia each year due to malaria infection(19).

According to WHO anemia global database published in 2008, it is estimated about 62.7% of pregnant women in Ethiopia are anemic that is indicative of the condition as a severe public health problem in the country(15). Recently, however, the prevalence of anemia is turning to be moderate public health problem in some areas of the country, such as 21.6% in Northwest Ethiopia(20) and 27.9% in Southeastern Ethiopia(21). The mild anemia is the most common type among pregnant women in the country(22).

Anemia among refugee pregnant women is one of the major health problem in many regions of the world(23). In 2014 from the total of 2,119,937 refugees in Africa the general anemia prevalence ranged from 15.2% in Tongo, Ethiopia to 84.4% in Bududuram, Ghana(24). From study conducted in eight South Sudanese refugee camps in Uganda, about 36.3% anemia burden was seen among mothers(25). High prevalence of anemia was reported from the nutritional survey in Saharawi refugee camps in Tindouf-Algeria, where it affected 76.5% of the pregnant women(26). Generally, anemia is a key public health problem in refugee set-up mainly due to inadequate intake of micronutrients and high rates of infections related to crowded environments and sanitation problem(27).

Anemia is an indicator of both poor nutrition and healthcare services of a respective community(7). It brings many undesirable effects on different population groups. Anemic infants and children may suffer from impaired psychomotor development and coordination, impaired language development, low scholastic achievement, and decreased physical activity. Anemia during pregnancy is responsible for increased feto-maternal morbidity and mortality, and high risk of LBW that can continue into the next generation(5-7). It is responsible for over 100,000 (about 20%) annual maternal deaths worldwide(11). Maternal anemia is associated with a twofold and threefold increased risk for pre-term delivery and a delivery of a LBW infant, respectively(5).

Economic loss due to anemia, specifically iron deficiency anemia, is estimated at approximately $2.32 per capita or 0.6 % of Gross Domestic Product (GDP), and this figure even rises to $16.78 per capita or 4.05 % of GDP if cognitive losses are considered(11). Appropriate diagnosis and treatment of anemia is capable of restoring personal health and raising national productivity levels by as much as 20%(28).

Despite efforts to reduce and control anemia burden in refugee population, the problem remains very serious mainly affecting young children and women. According to the report of Helen Keller international annual nutrition survey conducted in 2009, anemia prevalence was 28.9% and 38.3% among children and pregnant women, respectively, in Myanmar refugees in Bangladesh (18). These figures indicate the need to strengthen efforts for prevention, case detection and treatment of refugees in areas where high prevalence is detected. Preventing and reducing anemia among refugees demands a multi-dimensional and comprehensive approach: inline to this, anemia prevention and reduction was made an integral part of the United Nations High Commissioner for Refugees (UNHCR) Nutrition and Food Security Strategic Plan for 2008-2012. The UNHCR allocated about two million US dollars budget to halt the anemia burden in refugee population living in Ethiopia for the years 2008 to 2010(27). Nevertheless, the burden of anemia

has remained high in the refugee camps lacking any significant improvement. This was witnessed by report from Dollo Ado Nutrition Surveys in March 2013 in which the level of anemia among the children 6 to 59 months persisted higher than 40% while remained above 20% among women(29).

Conflict and other related conditions that lead people to migration disrupts the wellbeing of pregnant women. Globally about 59% of maternal deaths occur in fragile states, many of which are affected by conflict and recurring natural disasters. Pregnant women fleeing conflict lack the routine antenatal care (ANC) and may even die as they give birth on the run without assistance from a skilled health professional. A disaster or conflict hinders the accessibility of nutrients to pregnant women thereby predisposing them to the risk of anemia(30). Anemia is among the major causes of maternal deaths in refugees: it was responsible for 55% of the total deaths in Daddab refugee camp of Kenya in 2008(31).

Due to the recent crisis in South Sudan, over 447,000 people have fled to the neighbouring countries: Ethiopia, Kenya, Sudan and Uganda. Almost half of these people have joined the refugee camps in Ethiopia, and this huge influx raised the total number of refugees to 629,718 by the end of July 2013 making the country the biggest refugee hosting nation in Africa. Over 70% of the refugee population are children and women who have reduced capability of supporting themselves(32).

People living in refugee camps can suffer from different kinds of difficulties: nutritional deficiency is among the leading problems for which pregnant women and children are more vulnerable(33). Anemia is one of the nutritional problems that is commonly encountered by these community members in refugee setting, and it was found to be severe public health problem among under-five children in refugee camps of Gambela (>40%)(34). This figure alarms to assess the burden of anemia and associated factors among pregnant women for whom specific research data is unavailable despite their vulnerability to the existing socio-demographic strain and heavy malaria burden in the area. Hence, a particular data for pregnant women showing the burden along with contributing factors is desperately needed to combat anemia in this vulnerable population groups.

CHAPTER TWO: LITERATURE REVIEW

2.1. Anemia

Anemia in pregnancy is prominent public health problem in many parts of the world irrespective of their economic status. In 2011, it was found affecting 38% and 22% of pregnant women in the world and high income counties, respectively(35). A cross-sectional survey conducted from November 2003 to May 2004 in East Anatolian province, Turkey, indicated 27.1% anemia prevalence among pregnant women. From this 27.1% total prevalence, 56.6% was morphologically normocytic-normochromic type(36).

The burden is worse when considered specifically in developing parts of the world. Countrywide survey conducted from 2002-2004 in India showed over 90% prevalence of anemia among pregnant women. According to this survey over half of this burden accounts for mild form of anemia, and about 40% and 3% of pregnant women were suffering from moderate and severe anemia, respectively(37). Thirty five percent of pregnant women in Malaysia were affected by anemia according to the result of multi-centered epidemiological survey conducted in 2005 consisting 1,072 pregnant women(38). Nowadays, the global anemia prevalence among pregnant women is showing a slow decline through time as it was seen from different studies conducted in 107 countries which was 43% in 1995 to 38% in 2011(35).

Africa is the continent where anemia affected majority of its pregnant women population(15). In 2012 from a cross-sectional study conducted on 300 pregnant women in Niger delta area of Nigeria, 66.7% anemia prevalence was found, from which 55.7% had mild and 44.3% had moderate anemia(39). The condition keeps being the headache of many areas across the continent according to reports from Kakamega County in Kenya (40%)(40), Uthungulu Health District in South Africa (57.3%)(41) and Sidi Bel Abbes Region in Algeria (40%)(42).

Being one of the developing countries, Ethiopia is among the top maternal anemia burden nations in the world as indicated by the results of different local and international studies: 62.7% from WHO(15), 27.9% from Southeast Ethiopia(21), 30.4% from nine Regions in Ethiopia(43) and 39.9% from Wolayta Sodo, Southern Ethiopia(44). According to the 2011 Ethiopian Demographic Health Survey (EDHS) result, 22% of pregnant women in the country were anemic(22). Normocytic-normochromic anemia was reported from Wolayta Sodo Town, Southern Ethiopia (75.17%)(44) and Gondar, Northwest Ethiopia (97.1%)(20).

Anemia is one of the frontline public health problem in refugee setup, particularly in pregnant women. In 2006, 38.6% anemia prevalence was observed among pregnant in Palestinian refugees, from which 92.4%

was mild type, and moderate and severe anemia collectively accounted for the remaining percentage(45). The post-arrival screening examinations on Bhutanese refugees in United States of America (USA) showed 28% anemia burden among pregnant women(46). From facility-based survey conducted in 2003 on Afghan refugee pregnant mothers living in Peshawar, Pakistan, 42.5% anemia burden was observed(47). Anemia is the problem of mothers in refugee camps of Africa as indicated by Food Security and Nutrition Assessment among South Sudanese Refugees in Uganda, where it affected 36.3% of the reproductive-age women(25).

2.2. Socio-demographic factors

Anemia is one of the most common problem among population groups with low socio-economic status(18). Socio-demographic and economic characteristics such as ethnicity, sex, age, occupation, educational status, marital status, and income play their role through depriving a person from acquiring and maintaining sufficient amount of micronutrients by different mechanisms. These factors have clearly put their effect on the observed 62.6% anemia burden among 227 pregnant women in River State, Nigeria, where anemia was significantly associated with educational status (P= 0.020) and economic status (P= 0.030) of study participants(48). Similar to this finding, pregnant women from lower economic class carried higher prevalence of anemia (79.1%) compared to those from higher economic class (20.9%) in Kiboga district of Uganda(49).

Educational status of a woman has a big role in the anemia occurrence according to findings from different studies. A study conducted in India showed higher burden of anemia among those lacking education at all (82.4%) than those with any educational level, (57%)(50). Occupation of a women also affects the anemia prevalence in pregnant women, and this fact was supported by the finding from Gondar Hospital ANC followers, where housewives were twice more likely to develop anemia than those of government employees(51). Age of the woman has a big contribution to anemia occurrence. A highly significant association between mother's age and anemia was found from a facility-based study in rural area of India ($\chi 2=28.38$, $P < 0.001$)(50).

2.2.1. Conflict/migration related factors

Armed conflict is one of the major causes of food and nutrition insecurity by disturbing the normal production, distribution network and the marketing system in that country. It affects women and children severely relative to other population groups forcing them to be refugees, and it is estimated that about half

of refugee population are women(52). The complex experiences of pregnant women in refugee settings have a major impact on their health. Primary healthcare services in the refugee setups are believed to be inadequate to address the specific needs of pregnant women(14).

Nutritional status of refugees can be compromised mainly due to dislocation, lack of income, and limited access to nutritious foods(53). Pregnant women require additional nutrition, but in many conflict situations it is a big headache to meet these needs. Hence, spending longer time in this situation that has limited access to healthcare and nutrition rises the risk of micronutrient deficiency(54). Migration exposes pregnant women to anemia by compromising availability of essential micronutrients and obliging them to live in slum condition (i.e., poor access to water, sanitation and healthcare) that may lead to infections(55).

2.3. Obstetric factors

Gestational age, parity, time gap between consecutive births, history of excess menstruation and blood loss during current pregnancy are among the most important obstetric and reproductive related factors responsible for the occurrence of anemia during pregnancy(10,19). High number of children was shown predictor of anemia among pregnant women in many studies as it eventually drains iron store(44,56-59).

Result of studies conducted in urban area of Eastern Ethiopia and Palestine has shown increment of the anemia burden parallel to the climb in gestational age(45,60). In study from urban area of Eastern Ethiopia, pregnant women in second and third trimester were 2.87(95%CI: 1.61-5.17) and 3.32(95%CI: 1.84-6.0) times more likely to be anemic, respectively, as compared to those in the first trimester. Third trimester increased the risk of having anemia by 11.37(95%CI: 4.56-24.82) among pregnant women in study done in Wolayta Sodo Town, Southern Ethiopia(44). Menstruation persisted for longer than five days and birth interval less than two years had increased the likelihood of anemia in studies conducted in Wolayta Sodo Town, Southern Ethiopia and Tikur Anbessa, Addis Ababa Ethiopia, respectively(44,56).

Blood loss during the current pregnancy and history of abortion were among the responsible factors for occurrence of anemia in pregnant women. Over sixty-percent of pregnant women who encountered hemorrhage during pregnancy period were found to be anemic as indicated by study from Fayoum Governorate in Egypt(58). Similarly, bleeding during pregnancy had raised the risk of anemia by 1.67 among pregnant women in the study done in Tikur Anbessa, Addis Ababa Ethiopia(56).

2.4. Parasitic infection factors

2.4.1. Malaria infection factors

Malaria is an infectious disease characterized by attacking of RBCs by plasmodium parasite, and it is transmitted from infected person to another by female *Anopheles mosquitoes*. It causes anemia by destruction and removal of parasitized RBCs, shortening the life span of non-parasitized RBCs, and decreasing erythrocyte production in the bone marrow(6). Malaria threatens about 40% of the world's population lives, resulting in an estimated 300-500 million morbidity and over one million deaths per annum. Ninety percent of malaria cases in the world occur in Africa south of the Sahara desert(62). Each year, more than 30 million African women in malaria-endemic areas become pregnant and face the risk of P. *falciparum* infection, which is the major plasmodium species leading to anemia(13).

Pregnant women are more vulnerable to malaria attack due to the alteration in the immune system, in which cell mediated immunity is suppressed in association to the pregnancy. Pregnant women are threefold more likely to suffer from malaria infection compared to their non-pregnant counterparts, and have a mortality rate from severe disease that approaches 50%. In malaria endemic areas, it is estimated that at least 25% of pregnant women are infected with malaria(12). In these areas, malaria during pregnancy is able to cause up to 10,000 maternal deaths per year mainly by resulting in severe anemia. It also indirectly accounts for about 8-14% of LBW and 3-8% of annual infant mortality in that area(13).

Findings of many researches showed the significant role played by malaria in bringing maternal anemia in malaria-endemic areas, where P. *falciparum* is the dominant specie(63-65). Malaria shared 53% responsibility from the observed 40% anemia prevalence among pregnant women in Enugu District of Nigeria(65). According to the report of study in Kisumu, Western Kenya, pregnant women infected by malaria had 1.37(95%CI: 1.25-1.49) higher risk of anemia(63). A community-based study conducted in Gilgel Gibe Dam area, Southwest Ethiopia, had shown the existence of strong association (adjusted odds ratio (AOR) = 11.19, 95%CI: 3.31-37.7)) between malaria and anemia among pregnant women. In this study, 93.3% of malaria infected pregnant women were found to be anemic compared to 48.7% of anemia burden among those free of malaria infection(66).

2.4.2. Intestinal parasites infestation factors

Intestinal parasitic infestation has a great ability to deplete micronutrients, particularly iron from the body and eventually leads to anemia. The world's leading cause of gastrointestinal blood loss is helminthiasis: like Hookworm infestation, which is common in many parts of the world(1,10). From a facility-based cross-sectional study conducted in Bangalore City, Southern India, 12.4% of the study population had helminthiasis from which 88.7% have developed anemia(67). The other facility-based cross-sectional study conducted in Enugu District in Nigeria showed the helminthes contribution to anemia: in this study Hookworm alone contributed about 27% for the overall 40% anemia burden among pregnant women(65). Similar finding was documented from study in Southeast Ethiopia, where intestinal parasitic infestation was found to be one of the predictors (AOR= 2.5, 95% CI: 1.3-4.8) for the observed 27.9% anemia prevalence among pregnant women(21).

Intestinal parasitic infestation is one of the common health problem loaded on refugee population in the world. About 18% of Palestinian refugees were suffering from different types of intestinal parasites in refugee camps of Gaza that may eventually lead to anemia(68).

2.5. Nutrition and dietary habit factors

Intake of nutritionally important diets with respect to anemia and the dietary habit have a tremendous effect on the occurrence of anemia in a respective population. Consumption and frequency of iron rich foods like meat, fruit and green leafy vegetables significantly reduces the risk of anemia(10). According to the study report from Fayoum Governorate in Egypt, pregnant women who consumed meat less than once per week were 2.13 (95%CI: 1.2-3.7) times more likely to develop anemia relative to those consumed it more than once per week(58). Similarly, in a facility-based cross-sectional study conducted in West Arsi Zone, Ethiopia, higher prevalence of anemia (39.8%) was observed among pregnant women who consumed meat less than once per week than those with better frequency per week (16%)(59).

Fruit consumption has shown an association with maternal anemia by playing a preventive role through enhancing absorption of non-heme iron(10). A facility-based cross-sectional study carried-out in Fayoum Governorate, Egypt on 381 pregnant women revealed that anemia prevalence among those consuming fruits less than four times a week was almost double (66.2%) than those who consumed it more than four times a week (33.8%)(58). Frequency of green leafy vegetables consumption had also established a significant association with anemia as reported from West Arsi Zone, Ethiopia(59).

Tea and coffee drinking with or immediately after meal greatly affects the proper utilization of dietary iron, particularly non-heme type, by interfering with its absorption. Especially, tea contains a compound called Tannin that depletes the free iron through chelating with ionic iron(10). According to the study done in Southwest Ethiopia in 2005, drinking tea/coffee immediately after meal leads to anemia. In this study 82.8% of pregnant women who drank tea/coffee immediately after meal on daily basis have developed anemia, in contrast to 4.7% burden among those who drunk it immediately after meal once a week(61). Taking tea always after meal increased risk of acquiring anemia by 12.3(95%CI:3.45-28.9) among pregnant women as reported by study from West Arsi Zone, Ethiopia(59).

The measurement of mid upper arm circumference (MUAC) has shown a clear association with maternal anemia(21,60). Study done in Urban area of Eastern Ethiopia showed that pregnant women with MUAC $\geq$ 23 cm had 59% lower risk of developing anemia compared to those with the MUAC <23 cm(AOR= 0.41, 95%CI: 0.27-0.63)(60). Likewise, MUAC was also identified as an independent predictor of anemia among pregnant women in a community-based cross-sectional study conducted in Westmoreland, Jamaica(69) and rural Sidama, Southern Ethiopia(70).

2.6. ANC service utilization factors

Pregnant women are at increased need for iron and folate than their non-pregnancy state to cope with their own and fetal requirement(8,10). Regular utilization of health services, such as attending ANC, taking supplements together with delivery at health facilities help to lower the risk of anemia among pregnant women by preventing iron deficiency(11,16). Iron deficiency is the major cause of nutritional anemia in the world(5). In Southern Asia, 56% of pregnant women suffer from anemia associated to nutritional deficiency, and iron deficiency takes the lion's share responsibility for the burden(71).

Failure to comply with the increased demand of these micronutrients leads pregnant women to eventually develop apparent anemia(8). During pregnancy in the absence of vitamin supplements, folate depletion can develop in up to 75% of pregnant women(10). Lack of iron supplement during pregnancy exposes a pregnant woman to anemia, and this fact was supported by a cross-sectional study done on pregnant women in Uganda where respondents who lacking iron supplements had 60% higher risk of developing anemia than their counterparts(72). Similar study done in Rural Sidama, Southern Ethiopia indicated the raise of anemia risk by 1.9(95%CI: 1.14-3.19) among pregnant women, who were not taking iron/folate supplement during the index pregnancy(70). Another supporting result was documented from study

conducted in Urban Area of Eastern Ethiopia: pregnant women missing iron supplement during pregnancy were 1.54(95%CI: 1.04-2.27) times more likely to develop anemia than who took it(60).

The number of ANC visit during index pregnancy was found to be significantly associated with anemia among pregnant women from studies done in Westmoreland, Jamaica(69) and Fayoum Governorate, Egypt(58). Lack of ANC service utilization in the previous pregnancy had increased the risk of having anemia by 1.11 in a facility-based study conducted in Tikur Anbessa, Addis Ababa, Ethiopia. This study has also revealed higher prevalence of anemia among pregnant women, who delivered their last baby at home (28.9%) compared to those delivered at health facility (22.0%)(56).

2.7. Conceptual framework

The conceptual framework was developed after reviewing many published literatures those shown the contribution of the factors discussed in literature review part for the observed anemia burden among pregnant women. These factors were grouped to give five major categories, namely: socio-demographic and economic, obstetrics and reproduction, parasitic infection, nutrition and dietary habit, and ANC service utilization related factors. Solid arrows indicate independent variables, which were reported as having association with anemia in other studies and they were assessed in this study. Broken arrows indicate the association that exist among those independent variables according to some literatures, but they were beyond the objectives of the current study (figure 1).

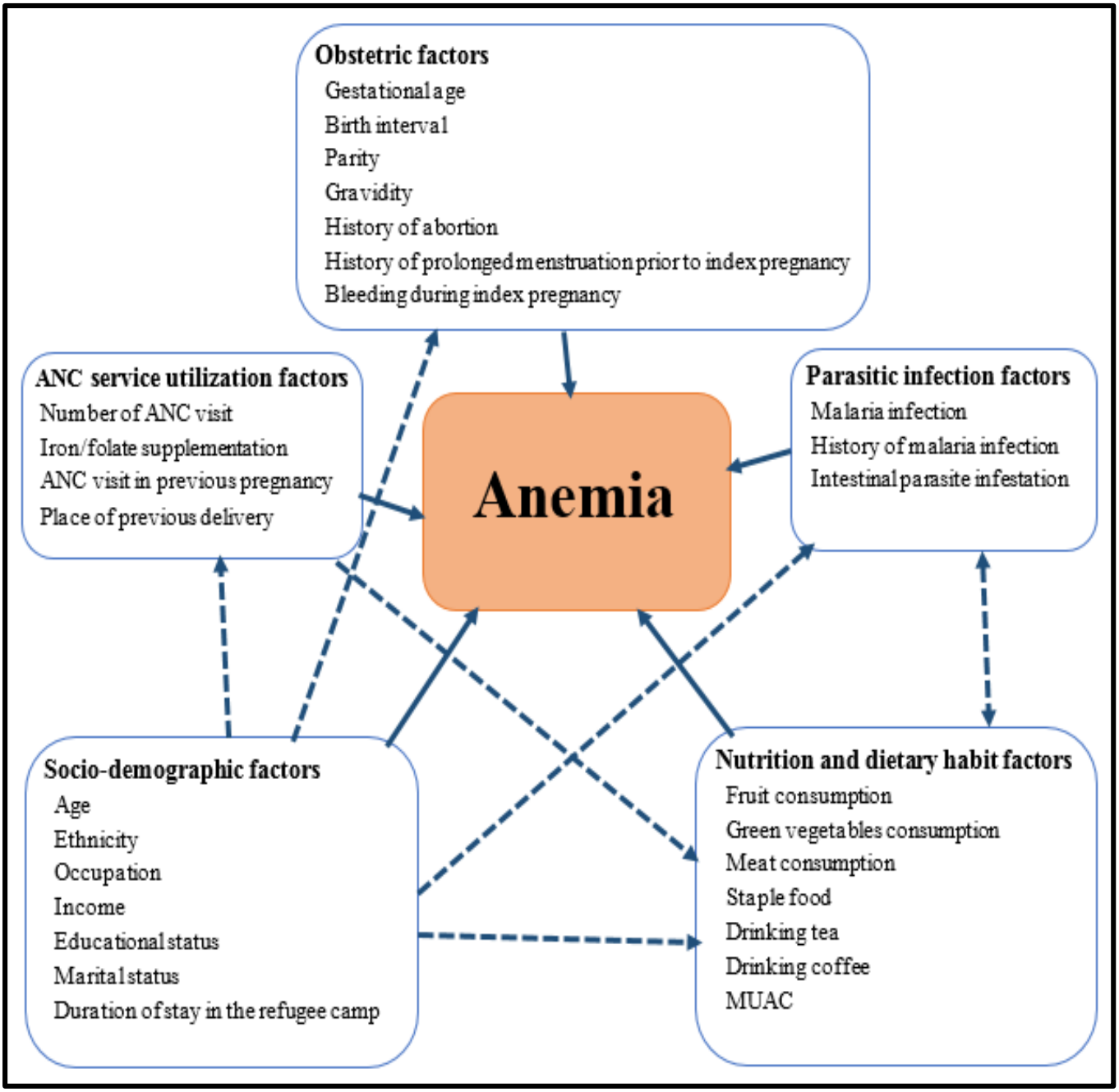

Figure 1: Conceptual framework of factors associated with anemia among pregnant women (prepared after reviewing related literatures)

2.8. Significance of the Study

Improving maternal health and reducing maternal mortality is a key concern of many local and international organizations. Recognizing its eminent impact, reducing anemia among women of reproductive age by 50% is the second global nutrition target for 2025. Given that, understanding the prevalence and associated factors is a critical component of any effort aimed at decreasing the burden of anemia among pregnant women. Findings of this study can provide a vital insight on the prevalence, severity and type of anemia along with associated factors in the respective population thereby pointing ways for designing specific and effective preventive mechanisms.

The management and control of anemia in pregnant women can be enhanced by the availability of local statistical data, which is, however, not adequately provided in our source population. The result obtained from this study can be used as a guide for health professionals working on pregnant women's care. It can also be used as an input for different policy makers in governmental and non-governmental organizations (NGOs) concerned in drawing strategic plan to halt anemia burden in this population. Findings from this study can be utilized as a reference for researchers interested to study similar issues in the area. It can also be helpful in improving the health status of the study participants by giving opportunity for diagnosis and treatment of anemia and parasitic infection in the facility free of any cost. Moreover, understanding specific health problems of refugee population not only improves the wellbeing of refugees in Ethiopia; but, it also ameliorates the overall public health status of the host community.

CHAPTER THREE: OBJECTIVES

3.1. General Objective

- To assess the prevalence, severity, type and associated factors of anemia among pregnant women in South Sudanese refugees attending antenatal care Clinic at Pugnido Administration of Refugee and Returnee Affairs (ARRA) Health Center, Gambela, Western Ethiopia, April-15 to June-30/2015.

3.2. Specific Objectives

- ✓ To assess the prevalence of anemia among pregnant women in South Sudanese refugees attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April-15 to June-30/2015.
- ✓ To determine the severity of anemia among pregnant women in South Sudanese refugees attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April-15 to June-30/2015.
- ✓ To determine the morphological type of anemia among pregnant women in South Sudanese refugees attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April-15 to June-30/2015.
- ✓ To identify the factors associated with anemia among pregnant women in South Sudanese refugees attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April-15 to June-30/2015.

CHAPTER FOUR: MATERIALS AND METHOD

4.1. Study area and period

The study was conducted from April 15 to June 30, 2015 in ANC department at Pugnido ARRA Health Center at Pugnido Town in Gambela Region, Western Ethiopia.

Pugnido Town is the capital of Gog Woreda, and located at a distance of 885 kms to the West from Addis Ababa and 110 kms from Gambela Town, the capital of Gambela Regional State. Gog Woreda is located on the Geographical coordinates of 7°35′ North Latitude and 34°15′ East Longitude, and it is bordered on the North by Abobo Woreda, on the South by Dimma Woreda, on the East by Abobo Woreda, and on the West by Jor Woreda and South Sudan. The altitude of the Woreda ranges from 400 to 600 m above sea level, and temperature ranges from 27 to 36 ^{o}C. Gog Woreda has a total population of 22,287 (projected from 2011 EDHS), from which 25.19% are urban inhabitants and majority of them belong to Anywa ethnic group. The Woreda has one district hospital (under construction), three governmental and two ARRA Health Centers, 12 health posts and three private Clinics(73).

Gog Woreda has one refugee camp near Pugnido Town comprising 54,597 refugees at the end of February 2015. The total number of pregnant women in the refugee camp was 3200 in the year. The livelihood of almost all refugee population is dependent on aid from different NGOs found in the region. Maize and wheat flour distributed by these NGOs, mango and fish available from the local market were the main dietary options consumed in the refugee camp(74).

4.2. Study design

A facility-based cross-sectional study design was used.

4.3. Population

4.3.1. Source population

All refugee pregnant women attending the ANC Clinic at Pugnido ARRA Health Center were considered as source population for this particular study.

4.3.2. Study population

Refugee pregnant women attending the ANC Clinic at Pugnido ARRA Health Center during the study period meeting the inclusion criteria were taken as study population.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

The inclusion criteria for this study were being pregnant women coming for the regular ANC visit, resident of Pugnido refugee camp and consented to participate.

4.4.2. Exclusion criteria

The study did not include pregnant women who were severely sick and those visited the Health Center for medical conditions (diabetes, chronic kidney disease and chronic hypertension), other than the regular ANC visit, for which follow-up is required. Pregnant women, who received blood transfusion in the last four months prior to data collection date were excluded from the study.

4.5. Sample size and sampling techniques

4.5.1. Sample size determination

The required sample size (n) was determined by using a single population proportion formula at a confidence level of 95% and 5% margin of error. We have considered 50% prevalence of anemia due to lack of previous studies about the prevalence of anemia focused at refugee pregnant women in this particular area and to have the maximum sample size. Thus, applying the following formula:

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

Where:
n= total sample size
z= confidence interval
p=prevalence of anemia
q= 1-p
d= margin of error

Equation 1: Formula for calculating sample size(75).

The initial sample size became 384. However, the number of pregnant women in this study community was <10,000, which was 3200, and therefore population correction formula was employed as follows:

$$n = \frac{n_0}{\left(1 + \frac{n_0}{N}\right)}$$

Where:
n_0 = 384 (Initial sample size)
N = 3200 (The number of pregnant women among the refugees in the year)
n = 343 (Corrected sample size)

Equation 2: Formula for calculating population correction in sample size determination(76).

Based on the correction formula the sample size became 343, and after adding 10% non-response rate the total sample size turned-out to be 377.

4.5.2. Sampling technique

By using convenient sampling technique, all pregnant women attending ANC Clinic at Pugnido ARRA Health Center during data collection period who met inclusion criteria and gave consent were included in the study.

4.6. Study variables

4.6.1. Dependent variable

- Anemia among pregnant women

4.6.2. Independent Variables

A. Socio-demographic variables

- Age
- Ethnicity
- Educational status
- Marital status
- Occupation
- Income
- Duration of stay in the refugee camp

B. Obstetrics variables

- Gestational age
- Parity
- History of abortion
- Bleeding in the current pregnancy
- Gravidity
- Birth interval
- Prolonged menstruation prior to the current pregnancy

C. Parasitic infection variables

- Malaria infection
- History of malaria infection
- Intestinal parasite infestation

D. Nutrition and dietary habit variables

- Meat consumption
- Green leafy vegetable consumption
- Fruit consumption
- Staple food
- Tea drinking
- Coffee drinking
- MUAC

E. ANC service utilization variables

- Number of ANC visit
- Place of previous delivery
- Current supplementation of iron/folate
- ANC follow-up in the previous pregnancy

4.7. Data collection instrument and procedure

4.7.1. Questionnaire

Data on socio-demographic factors (age, education, occupation, marital status and others), obstetric factors (trimester, parity, gravidity, history of excess menstruation and birth interval), parasitic infection factors, nutritional factors (staple food, intake and frequency of meat, green leafy vegetables, fruits, tea and coffee) and ANC service utilization factors of the pregnant women were collected using pre-tested, structured interviewer administered questionnaire. The questionnaire was adapted from EDHS 2011 and different related literatures, and it was initially prepared in English. The interview was conducted by two trained midwives capable of reading and writing languages of the refugees, and the response of each pregnant woman to every question was recorded on the questionnaire as per the pre-determined instructions(22) (annex II).

4.7.2. Anthropometric measurement

After the end of interview, MUAC measurement was done for each pregnant woman by midwives. The measurement was taken from middle of upper left arm using standard MUAC tape(77) (annex III part VI).

4.7.3. Laboratory investigation

Following completion of the interview and MUAC measurement, each pregnant woman was requested to give blood and stool specimens for the hematological and parasitological examinations, respectively. The laboratory investigation was proceeded after the necessary specimen was collected.

4.7.3.1. Complete blood cell count

Four millilitre (ml) of venous blood was collected using Ethylenediaminetetraacetic acid (EDTA)-vacutainer tubes, and CBC was done using CELL-DYN 1800 (Abbott Laboratories Diagnostics Division, USA). The hematological parameters measured include: RBC count, Hb concentration, hematocrit (Hct), MCV, MCH, MCHC and RDW(2) (annex III part I and II).

In this study, anemia in pregnancy was considered as Hb concentration below 11 g/dl. Regarding its severity, the anemia was defined as mild, moderate and severe if the Hb concentration is 10-10.9 g/dl, 7-9.9 g/dl and less than 7 g/dl, respectively(4). Regarding the type of anemia for all Hb levels below 11 g/dl, normocytic-normochromic if MCV was from 80 fl to 100 fl with MCHC from 32 g/dl to 36 g/dl, microcytic-

hypochromic if MCV was less than 80 fl with MCHC less than 32 g/dl and macrocytic-normochromic if MCV was greater than 100 fl with MCHC from 32 g/dl to 36 g/dl(2).

4.7.3.2. Blood film examination

Thick and thin blood films were prepared, stained by 10% Giemsa Stain for 10-15 minutes and examined microscopically to investigate the presence of hemoparasites, particularly malaria(78) (annex III part IV).

From the samples, whose Hb concentration was below 11 g/dl, thin blood films were prepared by wedge method within two hours of collection. These blood films were stained by Wright Stain, and examined microscopically for RBCs morphology to characterize type of the anemia(79) (annex III part III).

4.7.3.3. Stool examination

All pregnant women were oriented how to collect large teaspoonful size and contamination free fecal specimen, and given clean, leak proof stool cups with applicator stick to bring fresh stool specimen. After obtaining the stool specimen, examination for intestinal parasites was performed within 30 minutes. Two slides were prepared from each stool specimen using direct wet-mount and formol-ether concentration techniques. The slides were examined by two senior laboratory technicians using 10X and 40X microscopic objectives (78) (annex III part V).

4.8. Data quality assurance

The questionnaire was translated by experts to the refugee populations' language, Nuer and Anywa languages, and then back to English by another expert to check for any discrepancy. Pre-test was done on 5% of the sample size among pregnant women living in Itang refugee camp prior to the start of the actual study, and based on the pre-test result corrective actions were taken. Data collectors and supervisors were given a two days training on procedures of data collection and techniques of filling the questionnaire before the start of the actual study.

During MUAC measurement and laboratory investigation standard operating procedures (SOPs) were strictly followed. Supervisors monitored the activities for compliance and have taken corrective actions when necessary. Blind rechecking of blood film results reported by data collectors were done by supervisors. Reagents going to be used for the study were preliminarily checked for their storage condition, expiry date; and those needed preparation were prepared in line with manufacturer's instruction. The prepared reagents were checked by known blood film specimen. Assayed control materials: low, normal and high were run and delta check was done for CELL-DYN 1800 to check its reliability. The obtained data were entered twice into EpiData version 3.1 and discrepancies were corrected.

4.9. Data processing and analysis

Data were coded, cleaned and entered into EpiData version 3.1, and then checked for completeness and consistency. The data were exported to SPSS version 20 (SPSS, Chicago, IL, USA) for analysis. Descriptive analyzes such as frequency and mean were performed to summarize the socio-demographic, obstetric and reproduction, parasitic infection, nutrition, ANC service utilization related factors and laboratory findings. Kolmogorov-Smirnov test was done to check the normality of the data distribution.

The relationship between anemia and independent variables was investigated through bivariate analysis. In bivariate analysis, variables with a P-value ≤ 0.20 were selected as candidates for multivariable logistic regression analysis to identify significantly associated variables to anemia and control confounding variables. Multivariable logistic regression analysis was performed by enter method and model fitness was confirmed by Hosmer-Lemeshow test. Frequency tables and charts, odds-ratios, p-values and 95% confidence intervals were used to present the results of univariate, bivariate and multivariable logistic regression analyzes. For all statistical tests, P-value < 0.05 was considered statistically significant.

4.10. Ethical consideration

Ethical clearance was obtained from Jimma University College of Health Sciences Ethical Review Committee, and support letter was obtained from Jimma University. In addition to this, permission and support letters were collected from Gambela Regional Health Bureau and ARRA Regional Office, and copies of these letters were dispatched to the concerned organizations. There were letters at the hands of data collectors to show for those who want to assure our recognition.

The data collectors explained the aim of the study and data collection procedures to the pregnant woman who came for the routine ANC service and, asked her if she would like to participate in the study. Additionally, it was explained that there will not be any incentive for participation in this study and the interviewee has a full right not to be involved in the study or withdraw from the study at any time. Agreement of a pregnant woman to participate in the study was ascertained by her written informed consent. Consent of pregnant women under the age of 18 years was accompanied by their husbands. Finally, confidentiality was ascertained by anonymization of the data, and personal data was not disclosed beyond data collectors, supervisors and principal investigator without full willingness of study participant.

During the data collection process, pregnant women found having anemia and/or parasitic infection were referred to their clinician at the ANC department and received the necessary treatment free of any cost.

4.11. Operational definitions

Anemia during pregnancy: Hb concentration below 11 g/dl(4).

Mild anemia: Hb level from 10-10.9 g/dl(4).

Moderate anemia: Hb level from 7-9.9 g/dl(4).

Severe anemia: Hb level less than 7 g/dl(4).

Not public health problem: When the prevalence of anemia is 4.9% or less(4).

Mild public health problem: When the prevalence of anemia is 5.0-19.9%(4).

Moderate public health problem: When the prevalence of anemia is 20.0-39.9%(4).

Severe public health problem: When the prevalence of anemia is 40.0% or above(4).

Microcytic-hypochromic anemia: Hb level less than 11 g/dl, MCV and MCHC values less than 80 fl and 32 g/dl, respectively(2).

Normocytic-normochromic anemia: Hb concentration less than 11 g/dl, MCV value between 80 fl and 100 fl and MCHC value between 32 g/dl and 36 g/dl(2).

Macrocytic-normochromic anemia: Hb concentration less than 11 g/dl, MCV value greater than 100 fl and MCHC value between 32 g/dl and 36 g/dl(2).

Pregnant woman: A Woman whose pregnancy was confirmed by human chorionic gonadotropin hormone test and clinical examination at the study Health Center(22).

Primigravidae: A woman who is pregnant for the first time(22).

Multigravidae: A woman who has had two or more previous pregnancies(22).

Nulliparous: A woman who has never delivered a child(22).

Primiparous: A woman who delivered a child for the first time(22).

Multiparous: A woman who had delivered a child for more than once(22).

First trimester: A period during pregnancy that covers from conception through the end of 12^{th} week(22).

Second trimester: A period during pregnancy that covers from 13^{th} through the end of 28^{th} week(22).

Third trimester: A period during pregnancy that covers from 29^{th} through delivery(22).

Prolonged menstruation: Menstrual bleeding in a woman that persisted longer than her usual number of days (commonly beyond eight days) (22).

Refugee: A person who has been forced to leave his/her country to escape war or persecution and live in foreign country(29).

Resident of refugee camp: A pregnant woman, who has identification and ration card of ARRA and UNHCR, respectively(29).

Immediately after meal: Drinking tea and/or coffee within one hour after eating food(2).

Senior laboratory technician: Diploma in Laboratory Science with at least two years work experience(78).

Intestinal parasitic infestation: Presence of one or more intestinal parasite in stool examination(78).

Malaria infection: Presence of one or more plasmodium species in blood film examination(78).

4.12. Dissemination plan

The findings of this study will be presented to Jimma University College of Health Sciences; Department of Medical Laboratory Science and Pathology as a requirement for completion of MSc. in Clinical Laboratory Science specialty in Hematology and Immunohematology. It will also be disseminated to the Gambela Regional Health Bureau, ARRA and the source population through discussion with focal persons, direct mailing, reports, conferences, workshop and local media focused message transmission.

The finding will also be preserved in health institutions, Colleges, Public Libraries, concerned governmental and NGOs Offices and other places where it is needed. Furthermore, copy of this material will be preserved in Jimma University Library and publication Office, College of Health Science and CBE Office. Finally, strong efforts will be made to publish the findings of this study on internationally reputable journals.

CHAPTER FIVE: RESULT

5.1. Socio-demographic characteristics

Three hundred sixty pregnant women were enrolled in this study, making 95.24% response rate. The mean age of the study participants was 25.78 ± 6.6 years, and 253(70.3%) were above the age of 20 years with the minimum and maximum age of 16 and 42 years, respectively. Sixty three (17.5%), 7(1.9%) and 2 (0.6%) of the study participants were widowed, divorced and single, respectively (not shown in the table). Half of the study participants lack formal education, from which 113(62.8%) were unable to read and write; the rest 95(26.4%), 73(20.3%), 12(3.3%) have completed primary, secondary, and college and above levels, respectively (not shown in the table). Twenty nine (8.1%) of the respondents have job, and 73(20.3%) of respondents had monthly income that ranged from 100 to 700 Ethiopian birr (ETB). Pugnido refugee camp is mainly inhabited by Nuer population (63.9%). The length of time that respondents spent in this camp ranged from one month to twenty years with mean duration of 63 months (table 1).

Table 1: Socio-demographic characteristics of the refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15- June 30/2015 (n= 360)

Characteristics	Category	Total	Percent
Age in years	16-20	107	29.7%
	21-25	90	25%
	26-30	90	25%
	31-35	41	11.4%
	≥ 36	32	8.9%
Ethnicity	Anywa	130	36.1%
	Nuer	230	63.9%
Occupation	Student	85	23.6%
	House wife	122	33.9%
	Employed	29	8.1%
	Others*	124	34.4%
Income availability	Yes	73	20.3%
	No	287	79.7%
Educational status	Unable to read and write	113	31.4%
	Able to read and write	247	68.6%
Marital status	Married	288	80.0%
	Unmarried**	72	20.0%
Duration of stay in the refugee camp	< 18 months	148	41.1%
	≥ 18 months	212	58.9%

*: Respondents who are not engaged in any of the mentioned occupations during study period, **: Respondents who are not in a married state during study period, (i.e., single, divorced or widowed)

5.2. Obstetric characteristics

The mean gestational age of the study participants was 25.3 ± 7.5 weeks with minimum and maximum gestational ages of 10 and 41 weeks, respectively. From the total of 360 pregnant women in the study: 165(45.8%) were on their second trimester and 78(21.7%) were primigravidae. Considering parity, 238 (66.1%) of the respondents were multiparous. The mean birth interval was 29.8 months, with minimum and maximum of 18 and 84 months, respectively. It was also found that 51(14.2%), 41(11.4%) and 64(22.7%) of the participants had history of bleeding during the current pregnancy, prolonged menstruation and abortion, respectively (table 2).

Table 2: Obstetric characteristics of the refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15- June 30/2015 (n= 360)

Characteristics	Category	Frequency	Percent
Trimester	First trimester	34	9.5%
	Second trimester	165	45.8%
	Third trimester	161	44.7%
Gravidity	Primigravidae	78	21.7%
	Multigravida	282	78.3%
Parity	Nullipara	91	25.3%
	Primiparae	31	8.6%
	Multipara	238	66.1%
Birth interval*	≤ 24 months	115	48.3%
	> 24 months	123	51.7%
Blood loss during current pregnancy	Yes	51	14.2%
	No	309	85.8%
Prolonged menstruation**	Yes	41	11.4%
	No	319	88.6%
History of abortion*	Yes	64	22.7%
	No	218	77.3%

*: The percent was computed from the total cases in the characteristics

**: Prolonged menstruation is persistence of menstruation longer than eight days

5.3. Parasitic infection characteristics

The prevalence of intestinal parasitic infestation was 26.4% (n= 95), from which Giardia *lamblia* (28.4%) took the highest proportion. As the study area is one of malaria endemic parts of Ethiopia, majority, 215(59.7%), of the respondents had history of malaria attack in the last one year. In addition to that, the current study revealed 15.9% malaria burden among pregnant women. The P. *falciparum* (82.5%) is the most common species of malaria among malaria infected respondents (figure 2 and 3).

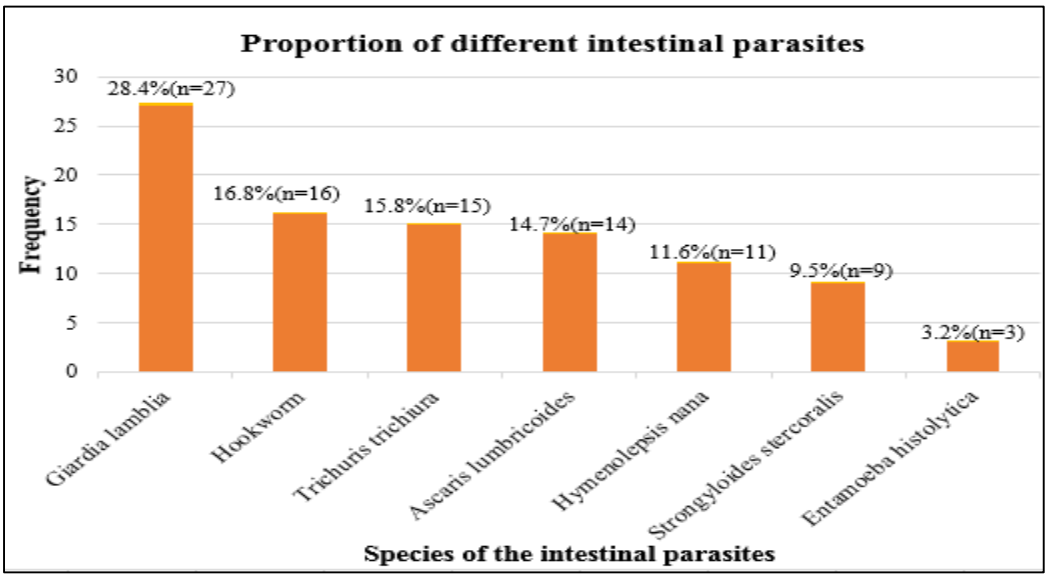

Figure 2: Proportion of different intestinal parasites among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15- June 30/2015 (n= 360)

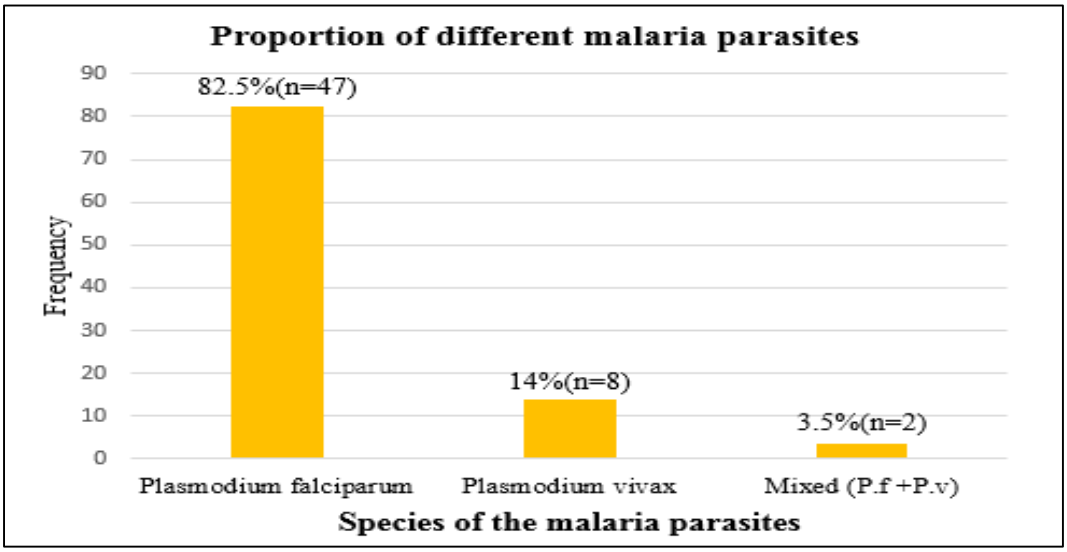

Figure 3: Proportion of different malaria parasites among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15- June 30/2015 (n= 360)

5.4. Nutrition and ANC service utilization characteristics

Majority of the pregnant women in the study area consume meat 321(89.2%), green leafy vegetables 325(90.3%) and fruits 272(75.6%). Porridge is the staple food of 249(69.2 %) pregnant women in the current study. Tea and coffee are among the most popular beverages consumed by the study participants, where 310(86.1%) of them drink tea and 255(70.8%) drink coffee. Mean value of MUAC measurement of the respondents was 22.9 ± 2.4 cm, with the minimum and maximum value being 16 cm and 30 cm, respectively (table 3).

Above three-fourth (n= 276) of the respondents were visiting the ANC Clinic for the first time, and there is no pregnant woman on fourth ANC visit. We have found low coverage of iron/folate supplementation in the study area as many, 291(80.8%), of the respondents did not receive the supplement during the current pregnancy. From the total of 269 child owning pregnant women, 146(54.2%) had delivered their last baby at home. One hundred fifty (55.7%) of the total 269 child owning study participants did not follow ANC service in the previous pregnancy (table 3).

Table 3: Nutrition and ANC service utilization characteristics of refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15- June 30/2015(n= 360)

Characteristics	Category	Frequency	Percent
Frequency of eating meat per week	At most once	192	53.4%
	More than once	168	46.6%
Frequency of eating vegetables per week	At most once	141	39.1%
	More than once	219	60.9%
Frequency of eating fruits per week	At most once	234	65.0%
	More than once	126	35.0%
Frequency of drinking tea immediately after meal per day	At least once	122	33.9%
	Less than once	238	66.1%
Frequency of drinking coffee immediately after meal per day	At least once	95	26.4%
	Less than once	265	73.6%
Staple food	Fish	44	12.2%
	Porridge	249	69.2%
	Injera	60	16.7%
	Other*	7	1.9%
MUAC value	< 21cm	68	18.9%
	≥ 21cm	292	81.1%
Number of ANC visit	One	276	76.7%
	≥ Two	84	23.3%
Taken iron/folate supplement**	Yes	69	19.2%
	No	291	80.8%
Place of previous delivery***	Health institution	123	45.8%
	Home	146	54.2%
ANC follow-up in previous pregnancy***	Yes	119	44.3%
	No	150	55.7%

*: Respondents those using bread and packed food as their staple food, **: Taken iron/folate supplement during index pregnancy, ***: The percent was computed from the total cases within the characteristics, MUAC: Mid-upper arm circumference, ANC: Antenatal care

5.5. Prevalence of anemia

The mean Hb concentration of the study participants was 11.3 ± 1.5 g/dl, with the minimum and maximum value of 6.2 g/dl and 17.5 g/dl, respectively. The average RBCs count, Hct, MCV, MCH, MCHC and RDW value of the participants were 4.15 x 10^{12}/l, 35.4%, 85.3 fl, 27.2 pg, 32.1 g/dl and 14.7%, respectively. One hundred thirty (36.1%) of the study participants' Hb value fell below 11 g/dl. Therefore, we found 36.1% prevalence of anemia, from which 89.2% (n= 116) is mild form. The most common morphological type of the anemia in the current study was normocytic-normochromic type, 56.1% (n= 73), (figure 4).

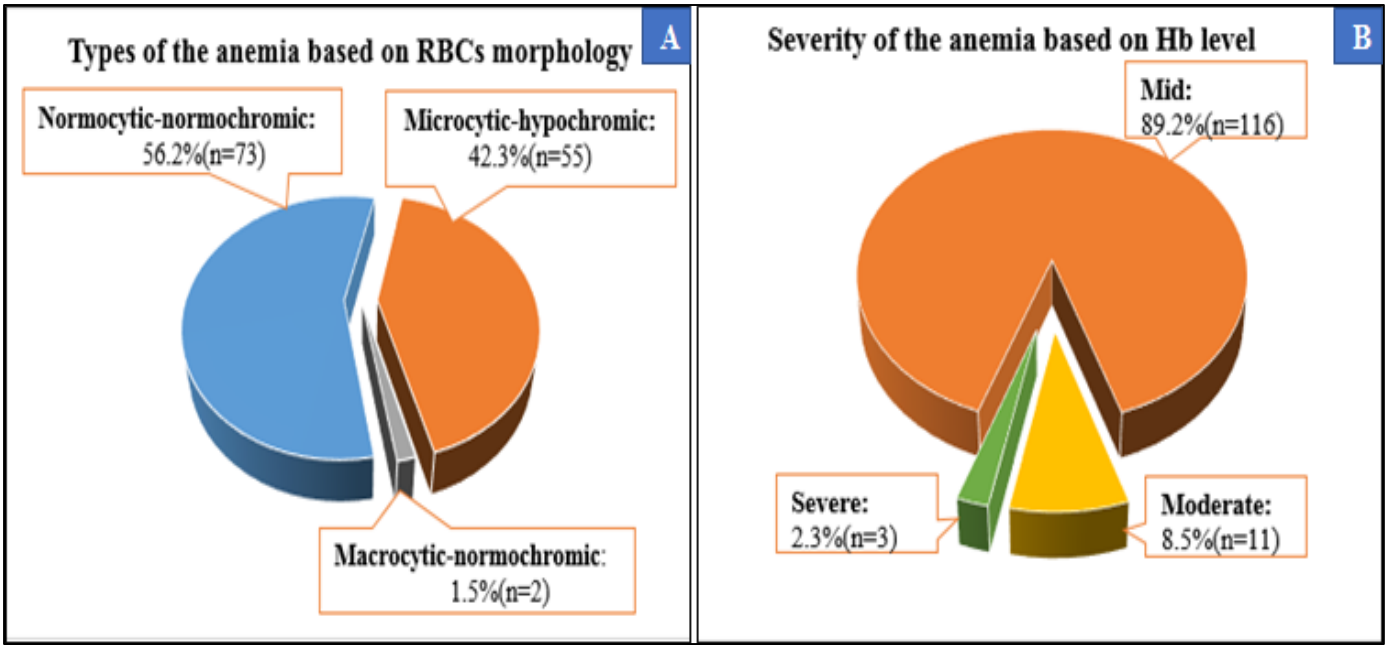

Figure 4: Proportion by morphological types (A) and severity (B) of the anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015 (n = 360).

5.6. Distribution of anemia with different characteristics

5.6.1. Socio-demographic characteristics and anemia

In the current study, prevalence of anemia was seen influenced by different socio-demographic and economic related aspects of pregnant women. Higher prevalence of anemia was observed among those who were above 35 years old (50%), employed (44.8%) and unmarried (37.5%) compared to their respective counterparts. Higher proportion of pregnant women, who were unable to read and write compared to literates (46.9% versus 31.2%) were affected by anemia. Similarly, pregnant women who spent less than 18 months in the refugee camp carried higher prevalence of anemia (43.9%) than those who lived for at least 18 months in the camp (30.7%) (table 4).

Table 4: Socio-demographic characteristics and anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015(n= 360)

Characteristics	Category	Anemia		Total	COR(95% CI)	P
		Yes n (%)	No n (%)			
Age in years	16-20	37(34.6%)	70(65.4%)	107	1.17(0.64-2.13)	0.606
	21-25	35(38.9%)	55(61.1%)	90	1.41(0.76-2.60)	0.175*
	26-30	28(31.1%)	62(68.9%)	90	1	
	31-35	14(34.1%)	27(65.9%)	41	1.15(0.52-2.52)	0.730
	≥ 36	16(50.0%)	16(50.0%)	32	2.21(0.97-5.05)	0.059*
Ethnicity	Anywa	48(36.9%)	82(63.1%)	130	0.95(0.60-1.48)	0.809
	Nuer	82(35.6%)	148(64.4%)	230	1	
Occupation	Student	26(30.6%)	59(69.4%)	85	1.29(0.72-2.33)	0.393
	House wife	46(37.7%)	76(62.3%)	122	0.94(0.56-1.58)	0.818
	Employed	13(44.8%)	16(55.2%)	29	0.70(0.31-1.59)	0.395
	Others**	45(36.3%)	79(63.7%)	124	1	
Income availability	Yes	32(43.8%)	41(56.2%)	73	1	
	No	98(34.1%)	189(65.9%)	287	0.66(0.39-1.12)	0.225
Educational status	Unable to read and write	53(46.9%)	60(53.1%)	113	1.95(1.23-3.08)	0.004*
	Able to read and write	77(31.2%)	170(68.8%)	247	1	
Marital status	Married	103(35.8%)	185(64.2%)	288	1	
	Unmarried***	27(37.5%)	45(62.5%)	72	0.93(0.54-1.58)	0.784
Duration of stay in refugee camp	< 18 months	65(43.9%)	83(56.1%)	148	1.77(1.14-2.74)	0.010*
	≥ 18 months	65(30.7%)	147(69.3%)	212	1	

*: Candidates for multivariable logistic regression analysis 1: Reference group, **: Respondents who are not engaged in any of the mentioned occupations during study period, ***: Respondents who are not in a married state during study period, (i.e., single, divorced and widowed)

5.6.2. Obstetric characteristics and anemia

Prevalence of anemia had shown a rise in line with increase in gestational age, number of pregnancy and number of children: from 17.6% in first trimester to 48.4% in third trimester, from 29.1% among primigravidae to 37.9% among multigravidae and from 33.0% among nulliparous to 37% among multiparous. Proportion of anemic pregnant women was higher among those who had maximum birth interval of two years (40.1%) than their corresponding counterparts. Likewise, anemia was more prevalent among those who had blood loss during current pregnancy (41.2%), prolonged menstrual bleeding prior to the current pregnancy (46.3%) and history of abortion (48.4%) (table 5).

Table 5: Obstetric characteristics and anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015(n=360)

Characteristics	Category	Anemia		Total	COR(95% CI)	P
		Yes n (%)	No n (%)			
Trimester	First	6(17.6%)	28(82.4%)	34	1	
	Second	46(27.9%)	119(72.1%)	165	2.43(1.53-3.85)	0.002*
	Third	78(48.4%)	83(51.6%)	161	4.38(1.72-11.16)	<0.001*
Gravidity	Primigravidae	23(29.1%)	55(70.5%)	78	1	
	Multigravidae	107(37.9%)	175(62.1%)	282	1.46(0.85-2.51)	0.170*
Parity	Nulliparous	30(33.0%)	61(67.0%)	91	1	
	Primiparae	12(38.7%)	19(61.3%)	31	0.78(0.33-1.81)	0.562
	Multipara	88(37.0%)	150(63.0%)	238	0.84(0.50-1.39)	0.498
Birth interval in months	≤ 24	45(39.1%)	70(60.9%)	115	1.19(0.70-2.02)	0.506
	> 24	43(35.0%)	80(65.0%)	123	1	
Bleeding during index pregnancy	Yes	21(41.2%)	30(58.8%)	51	1.28(0.70-2.35)	0.417
	No	109(35.3%)	200(64.7%)	309	1	
Prolonged menstruation	Yes	19(46.3%)	22(53.7%)	41	1.62(0.84-3.12)	0.150*
	No	111(34.8)	208(65.2%)	319	1	
History of abortion	Yes	31(48.4%)	33(51.6%)	64	1.75(0.99-3.08)	0.051*
	No	76(34.9%)	142(65.1%)	218	1	

*: Candidates for multivariable logistic regression analysis, 1: Reference group, ANC: Antenatal care

5.6.3. Parasitic infection characteristics and anemia

The current study revealed the difference in anemia prevalence with respect to parasitic infection. Higher prevalence of anemia was observed among pregnant women who were positive for malaria infection (52.6%) than those negative for the infection (33.0%). Previous malaria attack was seen to bring more than 15% difference in anemia prevalence: 26.9% among those who were not infected by malaria versus 42.3% among those who had the history in the last one year (table 6).

Presence of intestinal parasitic infestation was seen increasing the prevalence of anemia among pregnant women in the current study. The prevalence of anemia was substantially higher among pregnant women with intestinal parasitic infestation (50.5%) compared to those free of the infestation (30.9%) (table 6).

Table 6: Parasitic infection characteristics and anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015(n= 360).

Characteristics	Category	Anemia		Total	COR(95% CI)	P
		Yes n (%)	No n (%)			
History of malaria infection	Yes	91(42.3%)	124(57.7%)	215	1.99(1.26-3.15)	0.003*
	No	39(26.9%)	106(73.1%)	145	1	
Positive for current malaria infection	Yes	30(52.6%)	27(47.4%)	57	2.25(1.27-4.00)	0.005*
	No	100(33.0%)	203(67.0%)	303	1	
Positive for intestinal parasite infestation	Yes	48(50.5%)	47(49.5%)	95	2.28(1.41-3.68)	0.001*
	No	82(30.9%)	183(69.1%)	265	1	

*: Candidates for multivariable logistic regression analysis, 1: Reference group

5.6.4. Nutrition and ANC service utilization characteristics and anemia

Nutritional status and dietary habit of the pregnant women were found affecting the prevalence of anemia in the current study. Higher proportion of pregnant women were anemic among those who consumed meat (45.8%), and fruit (39.3%) once or less frequently per week than those with the higher frequency. Anemia was more prevalent among pregnant women who drunk tea (59.0%) and coffee (24.4%) immediately after meal at least once a day compared to their respective counterparts. We have found 73.5% anemia burden among pregnant women with MUAC below 21 cm, which is substantially higher than the burden among those with MUAC at least 21 cm (27.4%) (table 7).

Anemia prevalence was 35.1% among pregnant women who were visiting the ANC Clinic for the first time and did not received iron/folate supplement. Proportion of anemic pregnant women was higher

among those who had history of home delivery (40.4%), lacking ANC service in their last pregnancy (44.0%) (table 7).

Table 7: Nutrition and ANC service utilization characteristics and anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015(n= 360).

Characteristics	Category	Anemia		Total	COR(95% CI)	P
		Yes n (%)	No n (%)			
Freq. of eating meat per week	At most once	88(45.8%)	104(55.2%)	192	2.54(1.62-3.98)	<0.001*
	More than once	42(25.0%)	126(75.0%)	168	1	
Freq. of eating vegetable per week	At most once	46(32.6%)	95(67.4%)	141	0.78(.50-1.21)	0.269
	More than once	84(38.4%)	135(61.6%)	219	1	
Freq. of eating fruit per week	At most once	92(39.3%)	142(60.7%)	234	1.50(0.94-2.38)	0.085*
	More than once	38(30.2%)	88(69.8%)	126	1	
Staple food	Fish	19(43.2%)	25(56.8%)	44	1	
	Porridge	84(33.7%)	165(66.3%)	249	1.49(0.78-2.86)	0.228
	Injera	23(38.3%)	37(61.7%)	60	1.22(0.55-2.70)	0.619
	Other**	4(57.1%)	3(42.9%)	7	0.57(0.11-2.85)	0.494
Freq. of drinking tea immediately after meal per day	At least once	72(59.0%)	50(41.0%)	122	4.47(2.80-7.12)	<0.001*
	Less than once	58(24.4%)	180(75.6%)	238	1	
Freq. of drinking coffee immediately after meal per day	At least once	50(52.6%)	45(47.4%)	95	2.57(1.59-4.15)	0.001*
	Less than once	80(30.2%)	185(69.8%)	265	1	
MUAC	< 21 cm	50(73.5%)	18(26.5%)	68	7.36(4.05-13.37)	<0.001*
	≥ 21 cm	80(27.4%)	212(72.6%)	292	1	
No of ANC visit	1	97(35.1%)	179(64.9%)	276	1.20(0.72-1.97)	0.489
	≥ 2	33(39.3%)	51(60.7%)	84	1	
Taken iron/folate supplement***	Yes	28(40.6%)	41(59.4%)	69	1	
	No	102(35.1%)	189(64.9%)	291	1.26(0.74-2.16)	0.391
Place of previous delivery	Health institution	41(33.3%)	82(66.7%)	123	1	
	Home	59(40.4%)	87(59.6%)	146	0.74(0.45-1.21)	0.289
ANC follow-up in previous pregnancy	Yes	34(28.6%)	85(71.4%)	119	1	
	No	66(44.0%)	84(56.0%)	150	0.69(0.40-1.27)	0.188*

*: Candidates for multivariable logistic regression analysis, **: Respondents using bread and packed food as their staple food ***: Taken iron/folate supplement during the index pregnancy, MUAC: Mid-upper arm circumference, ANC: Antenatal care, Freq.: Frequency, 1: Reference group

5.7. Predictors of anemia

Multivariable logistic regression analysis was done for the variables with $P \leq 0.20$ in bivariate analysis to identify independent predictors of anemia. Sixteen variables complied with the criterion, and were analysed using multivariable logistic regression analysis by enter method. Finally being in third trimester, consuming meat at most once per week, drinking tea immediately after meal at least once a day, having MUAC below 21 cm and having intestinal parasitic infestation were found to be independent predictors of anemia among pregnant women.

The current study indicated that pregnant women existing in third trimester were 3.12 (95%CI: 1.16-9.83, P= 0.014) times more likely to be anemic than those in first trimester. Pregnant women who consumed meat at most once in a week had a twofold (95%CI: 1.11-3.58, P= 0.020) increased risk of developing anemia compared to those consumed meat more than once a week. Frequency of drinking tea immediately after meal at least once a day increased the likelihood of anemia by 3.01 (95%CI: 1.74-5.22, P <0.001) times compared to those who drunk less than once a day. The risk of developing anemia was raised by 3.90 (95%CI: 1.94-7.84, P <0.001) times among pregnant women whose MUAC was below 21 cm relative to those with MUAC of at least 21 cm. This study also revealed that pregnant women harboring intestinal parasites were 2.17 (95%CI: 1.20-3.91, P= 0.010) times more likely to be anemic than those free of the intestinal parasites (table 8).

Table 8: Multivariable logistic regression analysis result showing independent predictors of anemia among refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center, Gambela, Western Ethiopia, April 15-June 30/2015 (n=360)

Characteristics	Category	COR(95% CI)	P	AOR(95% CI)	P
Age in years	16-20	1.17(0.64-2.13)	0.606	1.64(0.67-4.04)	0.279
	21-25	1.41(0.76-2.60)	0.275	1.60(0.74-3.45)	0.230
	26-30	1		1	
	31-35	1.15(0.52-2.52)	0.730	0.75(0.28-1.99)	0.571
	≥ 36	2.21(0.97-5.05)	0.059	1.37(0.48-3.88)	0.547
Educational status	Unable to read and write	1.95(1.23-3.08)	0.004	1.46(0.80-2.66)	0.219
	Able to read and write	1		1	
Duration of stay in the refugee camp	Below 18 months	1.77(1.14-2.74)	0.010	1.04(0.57-1.91)	0.888
	At least 18 months	1		1	
Trimester	First	1		1	
	Second	2.43(1.53-3.85)	0.002	1.06(0.97-3.86)	0.056
	Third	4.38(1.72-11.16)	<0.001	3.12(1.16-9.83)	0.014*
Gravidity	Primigravidae	1		1	
	Multigravidae	1.46(0.85-2.51)	0.170	2.31(0.44-11.99)	0.320
ANC follow-up in the previous pregnancy	Yes	1		1	
	No	0.69(0.40-1.27)	0.188*	0.77(0.39-1.49)	0.340
Prolonged menstruation	Yes	1.62(0.84-3.12)	0.150	0.85(0.36-1.98)	0.712
	No	1		1	
Abortion	Yes	1.75(0.99-3.08)	0.050*	1.27(0.61-2.68)	0.519
	No	1		1	
History of malaria infection	Yes	1.99(1.26-3.15)	0.003	1.57(0.89-2.76)	0.118
	No	1		1	
Current malaria infection	Yes	2.25(1.27-4.00)	0.005	1.69(0.83-3.48)	0.149
	No	1		1	
Intestinal parasite infestation	Yes	2.28(1.41-3.68)	0.001	2.17(1.20-3.91)	0.010*
	No	1		1	
Freq. of eating meat per week	At most once	2.54(1.62-3.98)	<0.001	2.00(1.11-3.58)	0.020*
	More than once	1		1	
Freq. of eating fruit per week	At most once	1.50(0.94-2.38)	0.085	0.77(0.42-1.41)	0.407
	More than once	1		1	
Freq. of drinking tea immediately after meal per day	At least once	4.47(2.80-7.12)	<0.001	3.01(1.74-5.22)	<0.001*
	Less than once	1		1	
Freq. of drinking coffee immediately after meal per day	At least once	2.35(1.39-3.59)	0.001	0.54(0.29-1.02)	0.059
	Less than once	1			
MUAC	< 21 cm	7.36(4.05-13.37)	<0.001	3.90(1.94-7.84)	<0.001*
	≥ 21 cm	1		1	

*: Significant association at P < 0.05, AOR: Adjusted Odds Ratio, COR: Crud Odds Ratio, CI: Confidence Interval, Freq.: Frequency, MUAC: Mid-upper arm circumference, 1: Reference group

CHAPTER SIX: DSCUSSION

This study was aimed at determining anemia prevalence and its associated factors among South Sudanese refugee pregnant women attending ANC Clinic at Pugnido ARRA Health Center in Gambela region. From the total of 360 respondents, 130 (36.1%) were anemic. Based on WHO criteria, anemia was a moderate public health problem. Majority of the pregnant women were having mild and normocytic-normochromic anemia. Trimester of the pregnancy, frequency of meat and tea consumption, MUAC measurement and intestinal parasitic infestation were independent predictors of anemia.

The observed 36.1% anemia prevalence is nearly consistent with the study findings among pregnant women in Palestinian refugees in Occupied Palestinian territory (38.6%)(45), in Myanmar refugees in Bangladesh (38.3%)(18), mothers in South Sudanese refugees in Uganda (36.3%)(25), in Malaysia (35%)(38), in West Arsi Zone, Ethiopia (36.6%)(59) and in Southwest Ethiopia (38.1%)(61).

The prevalence in this study area is lower compared to findings from WHO in Ethiopia (62.7%)(15), Saharawi refugees in Algeria (76.5%)(26), Afghan refugees in Pakistan (42.5%)(47), Rural India (74.8%)(50), Eastern Sudan (62.6%)(80), Niger Delta, Nigeria (66.7%)(39) and Gilgel Gibe Dam area in Ethiopia (53.4%)(66). The possible reason for the observed lower prevalence may be due to methodological variation relative to studies from Gilgel Gibe Dam area(66) and Saharawi refugees in Algeria(26), which were community-based studies. Inclusion of larger number of participants in studies done in Peshawar, Pakistan(47) and Eastern Sudan(80) might also be among the methodological variation. In contrary to the current study, exclusion of those in first trimester from study done in Rural India(50) conceivably raised the prevalence there. The other sources of dissimilarity might be inclusion of pregnant women visiting the ANC clinic more than once in our study unlike the Niger Delta, Nigerian case that is restricted to those who were on their first visit(39). Time of the current study conducted might have undeniable role in lowering the prevalence: many NGOs were actively participating in different service provision, such as fortified food distribution.

The current study finding revealed higher anemia prevalence than the results of studies conducted in USA (28%) among pregnant women in Bhutanese refugee camp(46), East Anatolian Province, Turkey (27.1%)(36), Gondar, Northwest Ethiopia (16.6%)(51), Tikur Anbessa, Addis Ababa Ethiopia (21.3%)(56), Southeast Ethiopia (27.9%)(21) and EDHS (22%)(22). This difference could be due to better socio-demographic and economic status among pregnant women in Bhutanese refugees in USA, pregnant

women in Turkey and Gondar. The study area probably had its role in escalating the burden in our study in contrary to studies in urban areas: such as findings from Gondar and Tikur Anbessa, which are among the urban areas of Ethiopia. The exclusion of those who had chronic illness and antepartum bleeding in the study of Tikur Anbessa, Addis Ababa Ethiopia, unlike to the current study, might also have brought the difference. The current study consisted higher number of pregnant women in third trimester than the study in Southeast Ethiopia, which might have raised the prevalence. Generally, unlike many of the above studies, the use of cereal-based porridge as staple food and relatively higher burden of parasitic infection in this study setting might have increased the anemia burden.

The mild anemia was the common form of anemia in the current study, which accounted for 89.2% of the overall prevalence. The possible explanation for this finding could be the inclusion of pregnant women in the first trimester during which intensive hemodilution is not occurring and those received hematinics that help to prevent further fall in Hb concentration. This finding is concordant with the results of many studies conducted in different areas among pregnant women: Southeast Ethiopia (55%)(21), Tikur Anbessa, Addis Ababa Ethiopia (80.9%)(56), Kakamega County, Kenya (62.5% (38), Eastern Sudan (52.4%)(80), Palestine (92.4%)(45) and Krishna District in India (73.07%)(81), where mild anemia was common.

In contrary to our finding, moderate anemia was reported as the major form among pregnant women from similar studies in Wolayta Sodo Town, Southern Ethiopia (60%)(44), Karnataka, India (50.4%)(82) and West Algeria (49.5%)(42). The discrepancy against the study in Wolayta Sodo Town might have emanated from exclusion of pregnant women who were on hematinics unlike to the current study. Exclusion of pregnant women, who were in the first trimester and those visiting ANC for more than once in the Indian study might have contributed for the observed difference. The other possible source of inconsistency relative to study in West Algeria might be involvement of all pregnant women from third trimester. The prevalence of severe anemia among anemic respondents was 2.3%, which is almost similar to reports from Eastern Sudan (2.2%)(80), and Tikur Anbessa, Addis Ababa Ethiopia (1.19%)(56). However, this figure is considerably lower than study report from Rural India (18.9%)(50) that excluded pregnant women in the first trimester, which could possibly justify the discrepancy.

According to the RBCs morphological assessment, normocytic-normochromic anemia (56.2%) was the major type in this study area. Seventy three anemic pregnant women's red cells have normal size with normal Hb content. This might be due to the fact that inclusion of those with chronic infection, high prevalence of intestinal parasites and antepartum bleeding, which can result a fall in Hb concentration

while keeping the red cell indices within normal range. Our finding is in agreement with the study finding in East Anatolian province of Turkey (56.6%)(36), Kano Hospital in Nigeria (74.5%)(83), Wolayta Sodo Town, Southern Ethiopia (75.17%)(44) and Gondar, Northwest Ethiopia (76%)(51), where normocytic-normochromic red cell picture was seen among majority of anemic respondents. This morphological result is in contrary to results from studies done in Uyo University Teaching Hospital in Nigeria (66.5%)(84) and Krishna District in India(81), where microcytic-hypochromic blood cell picture was the most common. This inconsistency might have emanated from inclusion of pregnant women visiting the ANC Clinic more than once, who had received iron/folate supplement in our study.

Socio-demographic and economic factors can play a big role in determining the status of a pregnant woman with respect to anemia. These factors have different mechanisms to expose a pregnant woman to anemia: by increasing nutritional requirement in case of younger age, affecting knowledge about anemia and its prevention methods in case of education, affecting the ability to afford important nutrition and dietary habit in case of income availability together with educational status are some of the mechanisms. In our study, age of the respondents did not show significant association ($P > 0.05$) with the anemia. This finding is supported by previous studies in Eastern Sudan(81), Kiboga District in Uganda(49), Eastern Ethiopia(60) and Southwest Ethiopia(61). Contradictory findings were reported from studies in Palestine(45) and Tikur Anbessa, Addis Ababa Ethiopia(56), where significant association between anemia and age of pregnant women was seen. This variation might be due to use of larger sample size and presence of higher proportion of older age women in the study of Palestine and Addis Ababa, respectively.

Educational status, marital status, and ethnicity were not found to be independent predictors of anemia as witnessed by studies from areas of East Anatolian Province, Turkey(36), Mpigi, Uganda(72) Southeast Ethiopia(21) and Southwest Ethiopia(61). Despite the current finding, literacy status was indicated as significant factor for anemia in Bahrain(85), West Bengal, India(86) and Tikur Anbessa, Addis Ababa Ethiopia(56). The possible source of this disparity may possibly be the difference in study areas and composition of respondents in the study: the current study conducted in rural area with many participants unable to read and write unlike those in the above studies in which majority of participants were literate. Studies done in Malaysia(38) and Kisumu, West Kenya(63) have shown significant variation in anemia prevalence across different ethnic groups in contrary to the current study. This difference might be due to involvement of larger number of ethnic groups in these studies than ours that dealt with two groups only.

No statistically significant association was obtained between anemia and occupation, availability of monthly income and length of time spent in the refugee camp. In fact pregnant women spent longer time in the refugee camp carried lower prevalence of anemia. Studies conducted in Fayoum Governorate in Egypt(58), Southeast Ethiopia(21) and Gilgel Gibe Dam area in Southwest Ethiopia(66) supported our finding by reporting absence of significant association of anemia with occupation and income. Nevertheless, this finding is inconsistent with studies in Gondar, Northwest Ethiopia(51) and East Anatolian province, Turkey(36), where income became an important predictor for anemia. The discordance may be attributed to socio-economic difference between the areas: majority of participants in the present study lack monthly income unlike those in studies of Gondar and Turkey.

Generally, anemia occurrence in this study did not show any significant association with socio-demographic and economic characteristics. The possible explanation for this finding can be the similarity of the respondents as all of them were from the refugee camp, which is inhabited by population belonging to two ethnic group came from one country. More importantly, almost all of the refugees utilize similar services provided by aid organizations, which might narrow the gap among the participants with respect to these variables. This condition continued to affect other characteristics in the study making them to show nearly communal tendency in predicting the anemia than at an individual level.

Obstetrics and reproduction related characteristics of a pregnant woman play an important role in determining her Hb status through increasing requirement for iron or depletion of its storage(5). Despite this fact, many assessed attributes of the pregnant women that fall under this category did not show significant association with anemia. This finding is consistent with results of studies done in Rural India(50), Eastern Sudan(80), and Gondar, Northwest Ethiopia(51). The only exception was gestational age: pregnant women in the third trimester were 3.12(95%CI: 1.16-9.83; P= 0.014) times more likely to be anemic compared to those in the first trimester. This can be related to increased nutritional need for the rapidly growing fetus during third trimester. In line with our finding, third trimester was indicated as a predicting factor of anemia in Palestine(45), East Anatolian province, Turkey(36), and Wolayta Sodo Town, Southern Ethiopia(44). The current result was inconsistent with finding from Southeast Ethiopia(21) and Niger Delta Nigeria(39), where gestational age was not significant factor of anemia. The presence of relatively greater proportion of pregnant women in third trimester in the current study than those studies might have brought the difference.

Gravidity, parity and interval between successive births were indicated as predictors of maternal anemia in many studies(44,45,56,61) in contrary to the present finding. The possible reason for this deviation could be attainment of relatively longer birth interval among our respondents as compared to respondents in Tikur Anbessa, Addis Ababa Ethiopia(56). On the other hand, inconsistency with respect to parity and gravidity might be justified in relation to larger sample size and composition of relatively higher proportion of multiparous in Palestine(45) and multigravidae in Wolayta Sodo(44) studies. The current study has also revealed absence of significant connotation of anemia with history of abortion, excess menstruation and bleeding during index pregnancy similar to findings from different areas(51,59,85). However, contradicting result was reported from study done in Southeast Ethiopia(21), where history of heavy menstruation was reported as predictor of anemia. The prevalence of prolonged menstruation history among pregnant women in the current study was lower than in Southeast Ethiopia (11.4% versus 30.2%), and this might be the reason for this discrepancy.

Parasitic infection is among the leading causes of anemia through depletion of iron in the body and triggering suppression of erythropoiesis(6). We have assessed parasitic infection related aspects of the pregnant women; however, only having intestinal parasitic infestation was seen to increase the risk of developing anemia among pregnant women by 2.17 (95%CI: 1.20-3.91; P= 0.010). Similar to the current study finding, intestinal parasitic infestation was reported as a risk factor for anemia in pregnant women by results from studies done in India(67), Wolayta Sodo Town, Southern Ethiopia(44) and Southeast Ethiopia(21). Higher susceptibility of intestinal parasite harboring pregnant women to anemia can be due to ability of those parasites to cause gastrointestinal blood loss thereby orchestrating depletion of iron.

Malaria infection can cause anemia by triggering destruction of red cell(6), but in the current study we did not find any significant association between malaria infection (i.e., current and previous) and anemia. This finding is consistent with finding of the study from Tikur Anbessa, Addis Ababa Ethiopia(56), Southeast Ethiopia(21) and Gondar, Northwest Ethiopia(51). Despite the current finding, previous and current malaria infection has been indicated affecting the Hb concentration of pregnant women in other studies(20,63-66,80). This variation might be attributed to application of community-based study design, unlike to the current, in Gilgel Gibe Dam area(66) and Sidama Zone, Southern Ethiopia(64) studies. The other source of discrepancy might be relatively lower malaria prevalence than Kisumu, Western Kenya(63) and Enugu, Southeast Nigeria(65).

Nutritional insufficiency can expose people to plenty of undesirable conditions. Anemia is one of the foremost nutritional problems encountered by human being; particularly by those who are at increased need of the nutrient. Nutritional anemia develops if there is increased need accompanied by limited supply or difficulty in the utilization process of the specific micronutrient(4,10). Inadequate consumption and improper dietary habit with respect to the necessary micronutrient during pregnancy can increase the risk of developing anemia. The association of nutrition and dietary habit with anemia among pregnant women was revealed in several previous works(58-61,69,70,87); likewise three of the assessed nutrition related aspects of the respondents in this study displayed significant association with anemia.

Meat is one of the main sources of iron; and hence, its consumption frequency affects the status of a pregnant women with respect to anemia(10). Pregnant women, who consumed meat with a maximum frequency of once per week were two (95%CI: 1.11- 3.58; P= 0.020) times more likely to develop anemia than those with higher consumption frequency. In line with our finding, frequency of meat consumption was indicated as independent predictor of anemia among pregnant women from the studies done in West Arsi Zone, Ethiopia(59) and Fayoum Governorate, Egypt(58). Despite the current finding, study done in Tikur Anbessa, Addis Ababa Ethiopia(56) reported absence of significant association between anemia and meat consumption. The discrepancy might be related to presence of greater number of participants, who consumed meat in Addis Ababa study than the current study. This increased risk of anemia among those with low frequency of meat can be explained by the fact that meat is among the principal sources of heme iron, and hence its inadequacy leads to apparent anemia.

Tea contains a tannin molecule that inhibits the absorption of non-heme iron, and it considerably affect the absorption if taken immediately after meal(10). In the current study, drinking tea immediately after meal at least once a day increased the risk of anemia by 3.01(95%CI: 1.74- 5.22; P< 0.001) times among the respondents. This result is concordant with the findings from West Arsi Zone, Ethiopia(59) and Fayoum Governorate, Egypt(58). However, our finding was discordant with the study done in Gondar, Northwest Ethiopia(51), Tikur Anbessa, Addis Ababa Ethiopia(56) and Southwest Ethiopia(61). This dissimilarity might have originated from methodological variation: these studies tried to analyze the combined effect of tea and coffee on anemia unlike to the current study, which analyzed independently. This significant association might be due to the fact that frequent intake of tea immediately after meal has an inhibiting capability of iron absorption. This implies that keeping optimum gap between meal and tea intake helps prevent anemia by avoiding the formation of unabsorbable tannate complexes.

Nutritional status can be indicated by anthropometric measurements, such as MUAC. The MUAC has a big role in showing the nutritional status of a pregnant woman(77). In the current study, pregnant women having MUAC below 21 cm were 3.9(95%CI: 1.94-7.84; P< 0.001) times more likely to develop anemia than those with at least 21 cm. This finding is supported by similar studies from Rural Sidama, Southern Ethiopia(70) and Westmoreland, Jamaica(69), where MUAC was identified as predictor of anemia among pregnant women. This can be explained by the fact that pregnant women suffering from malnutrition are more likely to be micronutrient deficient including iron, which indisputably leads to anemia.

Absorption of non-heme iron is enhanced by the presence of green leafy vegetables and fruits in the food. These foods provide their components like ascorbic acid, which is essential for absorption of non-heme iron. Inadequate consumption of these foods can expose a pregnant woman for anemia(10). Relatively higher prevalence of anemia was observed among those with less frequency of vegetables (32.6%) and fruit (39.3%) consumption than those with the more frequency per week. However, except this prevalence difference, the current study did not reveal significant connotation of these factors with anemia. This finding is in line with studies done in Gondar, Northwest Ethiopia(51) and Southwest Ethiopia(61). In contrast to this study, a finding from Fayoum Governorate, Egypt(58) disclosed respondents with less fruit eating frequency per week had 1.9 times greater risk of developing anemia compared to their counterparts. This variation might have emanated from high fruit consumption, especially mango, in current study area.

Coffee contains caffeine that has an impeding effect on normal iron absorption, especially if no adequate time gap was kept between its intake and food(10). The current study showed the presence of higher prevalence of anemia among pregnant women with higher frequency of coffee intake immediately after meal (52.6%) than those with lower frequency (30.2%). However, no statistically significant association was established between anemia and frequency of coffee taken immediately after meal. Similar findings were reported from West Arsi Zone, Ethiopia(59) and Rural Sidama, Southern Ethiopia(70).

Iron deficiency may develop from failure to replenish increased utilization and loss of iron during pregnancy. Due to this, it is of a paramount to replenish the deficit by taking supplement together with properly utilizing the ANC services to survive the risk of anemia(16). Majority of the pregnant women were lacking iron/folate supplement due to low utilization of ANC service despite their raised gestational age. The number of ANC visit and iron/folate supplementation did not show significant association with anemia in the current study, and this finding is consistent with reports from study done in Gondar, Northwest Ethiopia(51). However, pregnant women who received iron/folate supplement and those with

better number of ANC visit were found to be better protected from risk of acquiring anemia as indicated by findings from Istanbul, Turkey(57) and Rural Sidama, Southern Ethiopia(70). This discrepancy might be due to low utilization of ANC service among majority of the respondents in the current study that can bring a significant prevalence difference.

Pregnant women, who did not follow ANC service in the previous pregnancy have increased risk of anemia in the index pregnancy as they lack iron/folate supplement, and health education concerning nutrition and related issues. Pregnant women lacking ANC service in the previous pregnancy carried higher prevalence of anemia (44.0%) than those followed it even though not statistically significant. In contrary to the current finding, lack of ANC service utilization in the previous pregnancy had increased the risk of anemia by 1.11 in a facility-based study conducted in Tikur Anbessa, Addis Ababa Ethiopia. The observed discrepancy might be due to low adherence to ANC service in the current study area. This study did not find significant association between anemia and place of last delivery, except apparently higher prevalence of anemia (40.4%) among pregnant women with history of home delivery. This finding is in line with the result from Tikur Anbessa, Addis Ababa Ethiopia(56). Generally, adverse pregnancy outcomes can be minimized if ANC is received early in the pregnancy and continued through delivery.

Strength and limitation of the study

Strength of the Study

We have tried to put the first effort on assessing and illuminating the problem of previously untouched community: refugee pregnant women in Ethiopia. Morphological assessment of RBCs was made, which is useful in identifying the type of anemia thereby suggesting ways for the possible underlining causes. Furthermore, we have tried to identify the predictors of anemia among pregnant women in this study.

Limitation of the Study

As the study design was cross-sectional; it is difficult to verify whether anemia preceded the predisposing factors or the vice versa in this study. Due to logistic constraints we did not measure serum ferritin, folate and cobalamin concentrations, which would help in specifically suggesting the micronutrient responsible for this anemia. The sampling technique used for selection of study participants might compromise representativeness of the result. We excluded severely ill pregnant women due to difficulty of getting venous sample, which may potentially reduce prevalence of anemia. The other limitation was lack of of similar studies especially among pregnant women in refugee setting to make comparative discussion.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

Anemia was a moderate public health problem in our study area. In the current study, normocytic-normochromic anemia was the common morphological type of anemia. Majority of the pregnant women in the study area had mild form of anemia. In Pugnido refugee camp, the prevalence of anemia among pregnant women remains high and is an area of priority. Given the detrimental consequences of anemia during pregnancy, this prevalence calls for urgent intervention.

Being in third trimester of the pregnancy heightened the susceptibility to anemia. This can be related to increased nutritional requirement during third trimester that cannot be met by food only, and hence the need for supplements.

The presence of intestinal parasitic infestation increased the risk of developing anemia among pregnant women. This might have emanated from the observed high prevalence of intestinal parasite in the area, which needs prompt control interventions and continuing attention.

Pregnant women consuming meat less frequently were at higher risk of developing anemia. More frequent consumption of meat would help to satisfy increased need for iron thereby reducing risk of anemia.

Frequent intake of tea immediately after meal significantly lowered the Hb level of respondents. Considerable number of pregnant women in this study have habit of drinking tea immediately after meal that can interfere with the normal absorption of iron. This signals a need for improving dietary habit.

Having low MUAC was seen contributing a big role in increasing the risk of anemia among the pregnant women. As it is a proxy indicator of nutritional status, we can conclude that a pregnant women having MUAC below 21 cm were suffering from a nutritional deficiency.

Generally, nutrition was identified as the major factor of anemia among pregnant women in the current study. From this we can conclude that huge maternal nutrition related problem is prevailing there, which can result substantial and lifelong effect on the mother and her child. Pregnancy is a critical time of human development, and anything that compromises fetal environment can have prominent and lasting effects on the child's future health. Our statement is strengthened by the current modification of long standing idiom "*you are what you eat*" into "*you are what your mother eats*" to emphasize the worth of maternal nutrition. Hence, keeping pregnant woman nutritionally healthy is improving general efficiency of the future generation.

7.2. Recommendation

Based on the current study findings, we have recommended the following for concerned bodies at all level.

- ARRA and Gambela Regional Health Bureau should work together to decrease the prevalence of anemia by integrating the identified factors to their anemia prevention and control policies.
- Health professionals working at ANC department of ARRA should aware pregnant women about the risk of anemia whenever the trimester of pregnancy rises and about the possible preventive measures.
- Gambela Regional Health Bureau together with ARRA, UNHCR and other NGOs working on maternal nutrition should stress on improving the dietary habit of pregnant women. Therefore, these concerned bodies are strongly recommended to give education to the pregnant women as many of nutrition and dietary habit related factors have raised the likelihood of anemia.
- Gambela Regional Health Bureau together with different NGOs needs to do a large-scale prevention to decrease the burden of intestinal parasites in the area.
- ARRA and Gambela Regional Health Bureau should make screening and treating of pregnant women for intestinal parasites as a routine practice to halt the possibility of eventually developing anemia.
- Researchers should do further large scale investigations in the area to identify specific nutrition related causes of anemia among pregnant women, particularly by assessing micronutrients (serum iron, folate and vitamine-B12 levels).

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ANNEXES

ANNEX I: Information sheet and Consent form

Part IA: Information sheet: English version

Jimma University College of Health Science Department of Medical Laboratory Science and Pathology

Data collector's name: _____ Code number: _____

Greeting!

My name is _____ and I am from Jimma University. I am conducting a research on anemia prevalence and associated factors among pregnant women in South Sudanese refugees attending ANC Clinic of Pugnido ARRA Health Center, and as part this you are kindly requested to participate in this study, which has a great importance in improving the community health status.

With this request, pregnant women who are attending ANC service at Pugnido ARRA Health Center and willing to participate in the study will be interviewed, and assessed by the laboratory investigation for anemia and parasitic infection. You have the right to accept, refuse or even stop participating in this study with no negative consequence. On the request paper your name or your identities will not be mentioned. The interview will take a maximum of 20 minutes and the laboratory investigation will take 40-60 minutes. You will be requested to give large teaspoon full amount of stool. Venous blood (4 ml) will be collected from the vein of your left arm using disposable syringe. There will be minor pain during blood collection but not harmful to your general health. If you are willing to give samples you will be requested to answer for question. No information concerning you as an individual will be passed to third party. Pregnant women found anemic and positive for parasitic infection will be treated in the facility free of any cost.

If you have any questions regarding this research you can use the following addresses to contact the concerned individuals.

Mr. Aklilu Alemayehu, phone no: +251917015610, Email address- aaakealex59@gmail.com

Dr. Tilahun Yemane, phone no: +251917804067, Email address- yemanetilahun@yahoo.com

Mr. Lealem Gedefaw, phone no: +251913024541, Email address- lealew07@gmail.com

Mr. Yaregal Asres, phone no: +251913724119, Email address- yaregala3@gmail.com

Are you willing to participate in this study by giving stool and blood sample, and answer to the questions?

Yes ☐ No ☐ ***Thank you!***

Part IB: Information sheet: Nuer version

Jimma University College of Health Science Department of Medical Laboratory Science and Pathology

Data collector's name: _____ Code: _____

Maale!

Ciōt dä cɔlä _____ oaa kä Jimmɛ University duel Gɔr. Kä hän oaa kɛ höö bä taa Daak riɛm been ciɛk kɛ beek dɛ kä mään tin ruet ni Tin mään ben kuic kä Pugnido rɔmjiith kɛɛm kä South Sudan duel wäl ARRA kä ɛkɛɛl ni qöör höö oaa quɔr kɛ höö ɛkɛɛl mibun kɛ kuic puɔlä puäNy naath.

Kɛ kuic thieckä mään tin ruetni laa tuum ruet kä quurɛ kä Kilinic ARRA kä hnɔkɛ Jɛ laa jaa quurɛ rɛy ŋiic kä nɛɛmɛ baa jɛ thieckä baajɛquic kɛ laboratory baa jɛ thiec kɛ Daak riem kɛnɛ Juath tin göödl. Nhɔki jɛ biɛ laat kɛ thiecni titi. Caa ciǒtdu bi laat kä caa bi Gɔr laat nikɛ boomŋ.

Thiecni titi bikɛ Jääncy kɛ kɔr minit ni 20 kä kɛ kuic laboratory minit ni 40-60 kä ji thiec kɛ höö bi riɛm thep kɛ nɛ we raaru kɛ kuic them kä baa riɛm kaan kä dɔmŋ tet kä du. mi kaan kɛ riɛm bɛ bɛɛc ɛmääth ciemi bi muoc kɛ riɛk. mi ciɛ hnɔk shöö bikɛ laat bä rɛɛt kä thiecni tin kɔmŋ. Thiɛlɛ riäät rami dɔmŋ mi baa laat rami dɔmŋ ciek miruet dɔmŋ deyɛ te daak riɛm kɛ juath Tin kɔmŋ ciet kɛ malaria laa kä muoc kɛ waal baamŋ.

Im ti kɛ thiec kɛ kuic rithɔackä nɔmɔ deri hää Jek kɛ Talipori ɛmɔ.

Mr. Aklilu Alemayehu, phone no: +251917015610, Email address- aaakealex59@gmail.com

Dr. Tilahun Yemane, phone no: +251917804067, Email address- yemanetilahun@yahoo.com

Mr. Lealem Gedefaw, phone no: +251913024541, Email address- lealew07@gmail.com

Mr. Yaregal Asres, phone no: +251913724119, Email address- yaregala3@gmail.com

Nhɔki jɛ bii läät kɛ nɛy kɛɛl kɛ höö biriɛm thep kiɛ we raaru; luoc thiecni day rɛw titi?

Hää ☐ Heey ☐

Ci laar tɛɛth lɔaac!

Part IC: Information sheet: Anywa version

Jimma University College of Health Science Department of Medical Laboratory Science and Pathology

Data collector's name: _____ Code: _____

Määdhë!

Cwödi mari nga_____ akälö Jïmma Unïvärcïtï. A tïïö bäät kwüänö mara thïn rïemø ki Jammi mo käl gø ki dïï män moa yïeth geda Jiey I Cøøth Cudan Repuji ki jiey mo ANC klinic i Pinynyuudo øt Jaath ARRA këël ki bäät Jamimi moi mi tïïc ii metec ibëët jööt dëël mar jiey.

Këël piëc moi mimo müm moa nyëëd ge ena mal cäädli kar ANC klinic mar øt jaath ARRA met ec kiper nee nut ge piëc mo ni da kwamø bang j labaratöri ni neetø thin rïemø ki twöngi mo käl täw. Jieri da teek ki man kweeri wala man jïey gø.

Bäät piëc cwödi mari ki mi tawkia patho manynyø piëc kädö ki digigemo 20 kere labaratöri nëëmö mari kädö 40-60 digige. ï da pëënyö ni cïepi ki rïemø mothin laac moa døøngø. Läyö mano käl rïemø ki bee cier caami ni dëël køny ki Baakötener nïïdel wala libïra wi lweeti thø dëël køny ki luancet.

Lieth mëëth pathadøc ni mäk mo ï net man ciëpï ki rïemø wala laac moa døøngø løny mam løgi pïec. Ba løny man tuume ni Jaami møøk dagø goa mäc rïemø mar ge thin ki Joa mäk ïeth ge da twonyi ge moø ki kiënë ki wat.

Ki pällë bärë nø acaan jïra man näk acaare moa cïba bëëdø nou ogwø ni bäng ngat ngäc gø.

Mr. Aklilu Alemayehu, phone no: +251917015610, Email address- aaakealex59@gmail.com

Dr. Tilahun Yemane, phone no: +251917804067, Email address- yemanetilahun@yahoo.com

Mr. Lealem Gedefaw, phone no: +251913024541, Email address- lealew07@gmail.com

Mr. Yaregal Asres, phone no: +251913724119, Email address- yaregala3@gmail.com

Ïïthu met man jiëyo bäät tïïc man ni rïemø da kälö ki laac moa døøngø, løy piëc bee?

Kare ☐ Patha kare ☐

Pøc jïïu!

Part IIA: Consent form: English version

Participant code number_____ Participant full name_____

I have clearly understood the aim of the research entitled "prevalence of anemia and associated factors among pregnant women in South Sudanese refugees in Pugnido" as I was informed in my own language, and therefore I am willing to participate in this study. I have been informed that medical history, blood and stool samples will be taken, and the presence of minimal pain during blood specimen collection. Furthermore, I was told about confidentiality of all information collected for the research.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Thank you!

Husband consent form

Participant code number_____ Husband full name_____

I have clearly understood the aim of the research entitled "prevalence of anemia and associated factors among pregnant women in South Sudanese refugees in Pugnido" as I was informed in my own language, and therefore I am willing to let my wife participate in this study. I have been informed that medical history, blood and stool samples will be taken, and the presence of minimal pain during blood specimen collection. Additionally, I was told about confidentiality of all information collected for the research purpose.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Thank you!

Part IIB: Consent form: Nuer version

Participant code number_____ Participant full name_____

hän cä Lot kä rithɔäc nɛɛmɛ ciɔl kɛɛ "taah Daak riɛm kɛ Tin kɔŋ Tin teekɛl kɛ Jɛ kä män Tin ruet kä South Sudan tin ci dääk tin tee kä Pugnidho", ciet kɛ mee caa laat dä kɛ thok da, kɛ kuic ɛmɔ, nhɔak kä Jɛ ɛnhöö dee nɛɛmɛ lät. Caa lät bä ɛnhöö, thiec ni ran kɛ kuic Juath riɛm kɛnɛ we raaru mitɔt baa kɛ naŋ kɛ bɛɛc in bɛc kɛ Jɛ mi ca riɛm mitɔt naŋ. Kɛ mindɔŋ cahä läät tääh in deeteekɛ mäc migɔa kɛ tääh in dee rithɔäc lät kɛ Jɛ.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Ci laar tɛɛth lɔaac!

Husband consent form

Participant code number_____ Husband full name_____

hän cä Lot kä rithɔäc nɛɛmɛ ciɔl kɛɛ "taah Daak riɛm kɛ Tin kɔŋ Tin teekɛl kɛ Jɛ kä män Tin ruet kä South Sudan tin ci dääk tin tee kä Pugnidho", ciet kɛ mee caa laat dä kɛ thok da, kɛ kuic ɛmɔ, nhɔak kä ciek dee nɛɛmɛ lät. Caa lät bä ɛnhöö, thiec ni ran kɛ kuic Juath riɛm kɛnɛ we raaru mitɔt baa kɛ naŋ kɛ bɛɛc in bɛc kɛ Jɛ mi ca riɛm mitɔt naŋ. Kɛ mindɔŋ cahä läät taah in deeteekɛ mäc migɔa kɛ tääh in dee rithɔäc lät kɛ Jɛ.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Ci laar tɛɛth lɔaac!

Part IIC: Consent form: Anywa version

Participant code number_____ Participant full name_____

Aani acara mar kwüänö man ni köö en ni “Døøn rïemø piny kit gii käl gø Jiey I Cøøth Cudan Repuji i Pinynyuudo” dät apook moa näk wälle ni näk ngät ge ena maal mo Cudään en thar piny aci wïïa ni wøp kaa mana caani jïra ki dhi-mara (Dha Agwaa). Kiper manøgø nø, aano jïëy ki man cïba acara mara kwüänö man. Aani jira man näk maa di pëënyö kiper täw manøgø rïemø ki laac moa døøngyøthwø okøbø ni di kalö, këël man dee rääm kanyo käl rïemø.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Pøc jïïu!

Aani acara mar kwüänö man ni köö en ni “Døøn rïemø piny kit gii käl gø Jiey I Cøøth Cudan Repuji i Pinynyuudo” dät apook moa näk wälle ni näk ngät ge ena maal mo Cudään en thar piny aci wïïa ni wøp kaa mana caani jïra ki dhi-mara (Dha Agwaa). Kiper manøgø nø, Dhaang paac ki man cïba acara mara kwüänö man. Aani jira man näk maa di pëënyö kiper täw manøgø rïemø ki laac moa døøngyøthwø okøbø ni di kalö, këël man dee rääm kanyo käl rïemø.

Signature: _____

Date: _____

Name of data collector: _____

Signature: _____

Date: _____

Pøc jïïu!

ANNEX II: Questionnaire

Part I: English version

Data collector's name: _____ Code: _____

Section I: Socio-demographic data

I01. How old are you? _____Years

I02. What is your ethnicity? Nuer ☐ Anywa☐ Other, ☐ Specify_____

I03. What is your occupation? Specify_____

I04. Do you have any income? Yes ☐ No ☐

I05. If the answer is yes for Q. no I04, what is your monthly income? _____

I06. What is your educational status?

Didn't attend any education ☐ Read and write ☐ Primary level (1-8) ☐

Secondary level (9-12) ☐ College and above ☐

I07. What is your marital status? Married ☐ Single ☐

Widowed ☐ Divorced ☐

I08. How long did you stayed in this refugee camp? _____

Section II: Obstetric data

II01. What is your gestational age? Probe and write in weeks ____

II02. Have you ever given birth? Yes ☐ No ☐

If the answer is yes for Q. No II02:

II03. How many children do you have? ____

II04. What is the average time interval between successive births? _____ Years

II05. Was there any blood loss during your current pregnancy? Yes ☐ No ☐

II06. Did you have any prolonged (longer than 8 days) menstrual bleeding prior to the current pregnancy?

Yes ☐ No ☐

II07. Did you have any abortion? Yes ☐ No ☐

Section III: Parasitic infection data

III01. Did you became infected with malaria in the last one year? Yes ☐ No ☐

Section IV: Nutrition and dietary habit data

IV01. Do you eat meat (after joining the refugee camp)? Yes ☐ No ☐

IV02. If the answer is yes for Q. No IV01, how often do you eat?

Every day ☐ Every other day ☐ Once a week ☐

Once a month ☐ Others, specify_____

IV03. Do you eat green leafy vegetables (after joining the refugee camp)? Yes ☐ No ☐

IV04. If the answer is yes for Q. No IV03, how often do you eat?

Every day ☐ Every other day ☐ Once a week ☐

Once a month ☐ Others specify _____

IV05. Do you eat fruits (after joining the refugee camp)? Yes ☐ No ☐

IV06. If the answer is yes for Q. No IV05, how often do you eat?

Every day ☐ Every other day ☐ Once a week ☐

Once a month ☐ Others specify _____

IV07. What is your staple food?

Injera ☐ Porridge ☐

Fish ☐ Other, specify _____

IV08. Do you drink tea? Yes ☐ No ☐
IV09. Do you drink tea immediately after meal? Yes ☐ No ☐
IV10. If the answer is yes for Q. No IV09, how often do you drink?

After every meal ☐ Once a day ☐
Every other day ☐ Occasionally ☐

IV11. Do you drink coffee? Yes ☐ No ☐
IV12. Do you drink coffee immediately after meal? Yes ☐ No ☐
IV13. If the answer is yes for Q. No IV12, how often do you drink?

After every meal ☐ Once a day ☐
Every other day ☐ Occasionally ☐

IV14. MUAC measurement _____ cm

Section V: ANC service utilization data

V01. Number of ANC visit; (observe the ANC card) ____
V02. Have you taken iron/folate supplement during the current pregnancy? Yes ☐ No ☐

V03. Where did you deliver your last baby? Health institution ☐ Home ☐

V04. Did you followed ANC service in your previous pregnancy? Yes ☐ No ☐

Thank you for your time and concern!

Part II: Nuer version Questionnaire

Section I: Socio-demographic data

I01.Tii rundi? _____ ruun

I02. ɛjin raan dōōrɛ miith? Nuer ☐ Anyuak ☐ Majaŋer ☐ Kiɛdōr tin kɔŋ ☐

I03. Laatdu ɛŋu? Laatɛ_____

I04. Tij miJeKi cet kɛ kuak tin Nyin Gooth kä raan? Hää ☐ Heey ☐

I05. mi ɛhää, ɛpek minindi LaaJeki mɔ?_____

I06. Ci Gɔaar kɛ pek minindi?

Kaan Gɔaar ☐ Kuenä kää Gɔaarä ☐ Cää Gɔaarkä 1-8 ☐

Cää Gɔaarkä 9- 12 ☐ Kā kɔlec ɛ we nhiam ☐

I07. Caa Ji kuɛɛn

Caa hää kuɛɛn ☐ Kaan hää kuɛɛn ☐

Ci cɔwdä liw ☐ Cää dääk ☐

I08. Tii rundii ka rɔml jiith? _____

Section II: Obstetric data

II01. Rueth ke pääth di? _____

II02. Ci daapni waal? Hää ☐ Heey ☐

mi ciɛ luoci ɛhää:

II03. Tii Gaat di? _____

II04. Laa Dōthi kɛ rundii? _____

II05. Rey ruetkädu mirueti te riɛmi laa lōōNy? Hää ☐ Heey ☐

II06. ɛŋuoti kääni ruet laa bɔth ɛlōōNy ɛlōŋ? Hää ☐ Heey ☐

II07. Ci Gaat met kääm raar? Hää ☐ Heey ☐

Section III: Parasitic infection data

III01. Ci malaria Ji met kääp? Hää ☐ Heey ☐

Section IV: Nutrition and dietary habit data

IV01. Laa mithi kɛ riiŋ? Hää ☐ Heey ☐

IV02. mi ciɛ luoci ɛhää; mithi di?

Niciaaŋ ☐ Thaaŋ quathni ☐

Ke Juok kä kɛɛl ☐ Kɛ pay kä kɛɛl ☐

IV03. Laa mithi kɛ jiith Juaacni? Hää ☐ Heey ☐
IV04. mi ciɛ luoci ɛhää; mithi di?

Nicia<u>a</u>ŋ ☐ Tha<u>a</u>ŋ quathni ☐

Ke Juok kä kɛɛl ☐ Kɛ pay kä kɛɛl ☐

IV05. Laa mithi kɛ dɛɛy Jiɛn? Hää ☐ Heey ☐
IV06. mi ciɛ luoci ɛhää; mithi di?

Nicia<u>a</u>ŋ ☐ Tha<u>a</u>ŋ quathni ☐

Ke Juok kä kɛɛl ☐ Kɛ pay kä kɛɛl ☐

IV07. Kuän ɛnhiam kɛ yiɛ ɛŋu?

Injera ☐ Kuän dääk ☐ Walwal ☐

Kōōp ☐ Tin kɔŋ , _____

IV08. Laa mää<u>t</u>hi kɛ c<u>aa</u>y? Hää ☐ Heey ☐
IV09. Laa maalɛ mää<u>t</u>hi kɛ c<u>aa</u>y niɛ täämɛ? Hää ☐ Heey ☐
IV10. mi ciɛ luoci ɛhää; micää kɔn miith?

Nicia<u>a</u>ŋ ☐ Kä kɛɛl cäry ☐

Tha<u>a</u>ŋ quathni ☐ Micää cää gōōr ☐

IV11. Laa mää<u>t</u>hi kɛ Bun? Hää ☐ Heey ☐
IV12. Laa maalɛ mithi kä mää<u>t</u>hi Bun? Hää ☐ Heey ☐
IV13. mi ciɛ luoci ɛhää; micää kɔn miith?

Nicia<u>a</u>ŋ ☐ Kä kɛɛl cäry ☐

Tha<u>a</u>ŋ quathni ☐ Micää cää gōōr ☐

IV14. MUAC measurement _____ cm

Section V: ANC service utilization data

V01. Nyuuth ni hä kaat tumindu? _____
V02. Te waal ti laa k<u>aa</u>m kɛ ruet ni kɛ kuic Gatkä ci kɛ kään? Hää ☐ Heey ☐

V03. Daapni, duel w<u>aa</u>l ☐ Cieŋ ☐

V04. Laa Tuum Ruet k<u>aa</u> Gouri (ANC) Hää ☐ Heey ☐

Ci l<u>aa</u>r tɛɛth lɔaac!

Part III: Anywa version Questionnaire

Section I: Socio-demographic data

I01. Mama bwötho I cöcïö? _____ cuiiri

I02. Jur mari ngøøni? Nwäär ☐ Anywaa ☐ Mijanger ☐ Kimøøk ☐ _____

I03. Tïïc mari ngøøni? _____

I04. Da Jamma mo joodi Kare ☐ Patha kare ☐

I05. Ni näk løk pïëc mari bee kare I04, Angøøni ojooti ki dwääy_____

I06. Yïno göödö?

Ï kär göödö ni bärë ☐ Kuääno ke göör ☐ Mana dikwøny (1-8) ☐

Riet ge (9-12) ☐ Kølech ki mal ☐

I07. Ï no nywømø? Jwöm marø nut ☐ Kär jwømø ☐

Cithøø ☐ Yïno bää ☐

I08. A cuïïrï dïï ni beeti i rupuji? _____

Section II: Obstetric data

II01. Yïno jaa ki pi dwääy? _____

II02. Yïnoni nyöötö? Kare ☐ Patha kare ☐

Ni mak løk pïëc mari kare II02:

II03. Obwöre dïï jïrï? _____

II04. Akøøt caae adïï ni cuïïrï yie? _____cuïïrï

II05. Rïemø da mo tumöni thiia ka gi ti ki dhaanhø muara anguudi? Kare ☐ Patha kare ☐

II06. Rïemø da mo cuïer ka gï tï ki dhaanhø muara anguudi ? Kare ☐ Patha kare ☐

II07. Da dhaanhø mari mo yïïno rämö ki gø? Kare ☐ Patha kare ☐

Section III: Parasitic infection data

III01. Yimo tuu ki täw maleri ki køør cuïïr aciel? Kare ☐ Patha kare ☐

Section IV: Nutrition and dietary habit data

IV01. Løny man cümi ki rïëngö? Kare ☐ Patha kare ☐

IV02. Ni näk kare IV01, akwärë adïï?

Ïcäng ☐ Ki køør nïnë ☐

Ki køør jwøøk ☐ Ki køør dwääy ☐

IV03. I camö ka marø këë lweet jiini? Kare ☐ Patha kare ☐

IV04. Ni näk kare IV03, akwärë dïï?

Ïcäng ☐ Ki køør nïnë ☐

Ki køør jwøøk ☐ Ki køør dwääy ☐

IV05. I cämö ki nyïï jiini? Kare ☐ Patha kare ☐

IV06. Ni näk kare IV05, akwärë dïï?

Ïcäng ☐ Ki køør nïnë ☐

Ki køør jwøøk ☐ Ki køør dwääy ☐

IV07. Wï cam mano cami amane?

Odëëna ☐ Rëëö ☐

Rïëng ☐ Kimøøk ☐, _____

IV08. Ï mäthö ki cay? Kare ☐ Patha kare ☐

IV09. Ï mäthö ki cay nii Køør cämö? Kare ☐ Patha kare ☐

IV10. Ni näk kare IV09, akwärë dïï oïthï?

Køør cam ☐ Ki køør cuïïrï ☐ Køør nïnë ☐ Kanyo manynyyi gø ☐

IV11. Ï mäthö ki buni? Kare ☐ Patha kare ☐

IV12. Ï mäthö ki buni nii Køør cämö? Kare ☐ Patha kare ☐

IV13. Ni näk kare IV12, akwärë dïï oïthï?

Køør cam ☐ Ki køør cuïïrï ☐ Ki køør nïnë ☐ Kanyo manynyyi gø ☐

IV14. MUAC measurement _____ cm

Section V: ANC service utilization data

V01. Kwörë moa cäädli ki gø bam kithi baath (neen kaat kithi baath) _____

V02. Yïïno kädö ki kïïnë moi ni nyrän pölët ni nyëë ti ena mal?

Kare ☐ Patha kare ☐

V03. Nyi laal mari inwøløka? Øt jaath ☐ Paac ☐

V04. Yïna cää thö bang kith bath (ANC)? Kare ☐ Patha kare ☐

Pøc jïïu!

ANNEX III: Standard Operating Procedures

Part I: SOP for venous blood collection

I. Materials and reagents

Tourniquet
Cotton wool
Vacutainer needle
Vacutainer tube holder
Alcohol
Disposable gloves
K_2EDTA vacutainer blood collection tube (Lavender)
Waste and sharps disposal containers

II. Phlebotomy procedure

1. Label tubes with the client's identification number.
2. Explain the blood drawing procedure to the client and reassure her
3. Wear the rubber gloves and make the patient a comfortable position
4. Prepare the vacutainer tube and needle
5. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5 cm to 10 cm above the puncture site
6. Tell the client to clench her fist
7. Using the tip of the index finger examine the phlebotomy site, feel the vein (preferably the median cubital), and decide exactly where to place the puncture.
8. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton wool soaked in alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
9. Insert the needle directly into the vein and draw 4 ml peripheral blood into K_2EDTA vacutainer tube
10. Tell the client to open her clenched fist.
11. Release the tourniquet.
12. Withdraw the needle from the vein and cover the puncture site with cotton swab and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate hemostasis is visible.
13. Properly discard the used materials in a safe container and tell the person to do so if handled the cotton swabs to stop the bleeding.
14. Mix the blood with the anticoagulant by gently inverting the tube 8 to 10 times.

Part II: SOP for complete blood cell count

I. Clinical significance

The test is used for diagnosis and monitoring of anemia, vascular integrity, leukemia and infectious diseases.

II. Principle

Blood sample is aspirated, diluted, mixed and enumerated based on electrical conductivity difference cells exhibit relative to the diluent, which is good electrical conductor. A suspension of blood cells passes through a small aperture simultaneously with an electric current. The individual blood cell passing through the aperture introduces an electrical impedance in the aperture proportional to its size. The system counts individual cells and provides the cell size distribution. The Hb measurement is done by using lytic reagent that causes lysis of the RBCs and conversion of the released Hb to a stable pigment whose absorbance is directly proportional to Hb concentration in the sample.

III. Specimen required

30 µl of whole blood collected into K_2EDTA anticoagulant vacutainer tube not older than eight hours.

IV. *Materials and equipments required*

CELL-DYN 1800 machine	Waste reservoir
CELL-DYN 1800 Diluent	CELL-DYN 1800 Controls
CELL-DYN 1800 Lyse	CELL-DYN 1800 Detergent
Biosafety materials	Enzymatic cleaner

Note, the reagents must be stored at room temperature except the enzymatic cleaner, which should be stored between 2 to 8 ^{o}C. Do not use reagents that have been frozen.

Procedure

Entering and running patient specimen

Note: prior to running patient specimens, perform daily start-up procedures

Press the **prime** option to make the machine ready

When **ready** message is displayed on the run screen, the instrument is ready to run specimens.

Entering specimen information

1. From **run** screen, press **[specimen type**]
2. In the specimen type screen, press **[patient specimen**]
3. The cursor is placed in the **<next id #>** entry field. Use the alphanumeric keys on the keyboard to enter a specimen identification code 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, mix the specimen by slowly inverting the tube at least 8 times
2. Remove the cap from the mixed specimen tube
3. Place the tube under the aspiration probe and raise tube so that the end of the probe is deeply immersed in the specimen
4. Press the run option on the touch plate to aspirate the sample
5. When the sample has been aspirated from the tube, the probe moves up; then remove the specimen tube and recap it
6. After the cycle is completed, run results are displayed on screen & aspiration probe moves into position to accept a new specimen. The current run data is saved to Data Log
7. If Automatic Graphics printout has been specified in the set-up menu, a report is printed according to the parameters selected during the set-up procedure
8. If automatic graphics printout has not been specified in the set-up menu, press **[print report**] to obtain a copy of the results

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

Quality Control

Quality control checks should be performed and quality control materials should be run as per the laboratory's protocol(79).

Procedure for QC

1- From the MAIN MENU screen, press [RUN]

2- From the RUN menu, press [SPECIMEN TYPE] followed by [QC TYPE].

3- Select the desired level of control (Low, Normal and High).

NOTE: Prepare a permanent record (printed copy) of any files to be selected or purged, as required, according to your laboratory protocol. You can also copy the QC Log before you purge it.

4- Using the arrows keys on the keyboard, select the desired control file.

5- Press [RETURN].

6- Remove the cap from a well-mixed control specimen tube and place the open tube under the sample aspiration probe. Raise the tube so that the end of the probe is deeply immersed in the specimen.

7- Press the Touch Plate to activate the run.

8- When the well-mixed control has been aspirated from the tube and the probe moves up through the Wash Block, remove the specimen tube and replace the cap.

9- Press [PRINT REPORT] if a report of printed results is desired.

Part III: SOP for blood film morphological analysis

I. Clinical significance

Blood film morphological analysis is important in the investigation and management of anemia, infections, and other conditions that produce changes in appearance of blood cells and differential white cell count.

II. Specimen required

Well mixed K_2EDTA anticoagulated whole blood not older than two hours

III. Materials and reagents

Frosted microscopic slides	Pencil
Alcohol	Disposable gloves
Microscope	Waste and sharps disposal containers
Wash bottle	Staining troughs
Slide forceps	Methanol
Wright Stain solution	Timer
Slide racks	Buffered water, pH 6.8, or distilled water

IV. Thin blood film preparation (Wedge method)

1. Use a glass slide that is free of dust, grease and debris

   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. Place a small drop of well mixed K_2EDTA blood (about 2-3 mm), 1.0 cm from the end of the glass slide, using either a plain capillary tube or other type of blood dropping device

   Note: When the blood is from anemic patient larger drop of blood should be used. If using an anticoagulated blood sample, make sure the specimen is well-mixed. The blood drop should be placed (opposite from the frosted end, if that type of slide is provided) and in the middle of the slide.

3. Place the spreading slide in front of the blood drop at an angle of about 30^o - 40^o to the slide and then move it back to make contact with the drop

4. The drop spreads-out quickly along the line of contact of the spreader with the slide. As the drop of blood spreads, be careful not to let it spread to both edges of the spreader

5. Advance spreader slide with a smooth steady motion so that a thin film of blood is spread over the slide

6. Allow the smear to air-dry

   Note: Do not blow on the smears as this can disrupt cellular morphology and causes formation of unwanted artifacts, like target cells; also do not use heat for drying.

7. Label the slide by the name of the patient and write the date or reference number on the head of the film using a lead pencil, a diamond marker or a graphite

V. Staining the thin blood film (Wright stain method)

Principle

Acidic dyes such as eosin unites with the basic components of the cell (cytoplasm) and hence the cytoplasm is eosinophilic (acidic), and basic stains like methylene blue or Azure B stain acidic parts of the cell (nucleic acids and nucleoproteins of the nucleus) and hence these structures are called basophilic (azurophilic).

Procedure

1. Place air-dried smear film side up on a staining rack (two parallel glass rods kept 5 cm apart).
2. Cover the blood film (preferably methanol pre-fixed for two minutes) with undiluted stain but do not flood the slide. If using a dropper bottle, count the number of drops required to cover the film.

   Note: The undiluted stain not only acts as a fixative but also partially stains the smear. This stage is required to obtain the best possible staining results. When an aqueous or diluted stain is used, the air dried smear must first be fixed by absolute methanol for 3-5 minutes

3. Add equal volume of pH 6.8 buffered water. The diluted stain should not overflow. Ensure the water is well mixed with the stain by blowing on the diluted stain or mixing the stain and water using a plastic bulb pipette until a metallic sheen appears. Allow to stain for 3-5 minutes.

   Note: Diluting the stain in buffered water brings about full staining of the blood cells. The exact staining time to use should be decided when a new batch of stain is prepared

4. Wash off the stain with tap water (filtered if not clean). Do not tip off the stain, because this will leave a fine deposit covering the film. Wipe the back of the slide clean and stand it in a draining rack for the smear to dry. The blood film should appear neither too pink nor too blue (check results microscopically).

Staining results

Red cells……Pink-red

Nucleus of cells ……Purple-violet

Cytoplasm

Neutrophils, eosinophils……...Pale pink

Monocytes……Grey-blue

Large lymphocytes……...Clear blue

Small lymphocytes……...Darker clear blue

Granules

Eosinophils…….Orange-red

Neutrophils…….Mauve-purple

Inclusions

Malaria pigment in monocyte....Brown-black

Howell-Jolly body…….....Purple-violet

Auer body (in myeloblast).....Purple-red

Basophils…….Dark blue-violet

Platelets……Purple-blue

VI. Microscopic examination of blood films

Every film should first be inspected at low power objective (10X) to check for even distribution of cells, staining quality, platelet clumping before general examination is undertaken with the 100X objective. It is essential to mount (cover) the film with a cover glass as this permits the film to be examined with the 10X and 40X objectives. Thus, when the film is completely dry cover it by a rectangular cover glass permanently with a neutral mountant (DPX). Survey the film at 10X magnification to get a general impression of its quality.

Find an area where the red cells are evenly distributed, just touching but not overlapping, and study their gross morphology at 40X objective; and at the same time, scan the film to get an impression of the quantitative distribution of WBCs. Identify any unusual or abnormal cells, estimate the relative proportion of platelets and note the presence of abnormally large platelets. Use the 100X objective for studying the fine details of the cell morphology.

VII. Reporting blood films

Reporting Romanowsky stained thin blood films includes:

–Differential white cell count and white cell morphology

–Red cell morphology

Report the appearance of the red cells, including:

–Variations in red cell staining, e.g. hypochromic cells, polychromatic cells, dimorphic blood picture. If normal, refer to the red cells as normochromic.

–Variations in red cell size, e.g. microcytes, macrocytes, spherocytes, significant anisocytosis. If normal in size, refer to the red cells as normocytic.

–Cells of abnormal shape or form, including pencil cells, sickle cells, target cells, fragmented cells (schistocytes), tear drop cells, 'bite' cells, burr (echinocytes) cells, poikilocytosis and cells showing rouleaux.

–Cells with inclusions, e.g. nucleated RBCs, megaloblasts, cells having Howell-Jolly bodies

–Comments on platelets (if venous EDTA anticoagulated blood sample is used)

–Microorganisms, e.g. presence of plasmodium, trypanosomes, microfilariae, and Borrelia

VIII. Quality Control

When a new batch of stain is prepared, decide the best staining time to use, e.g. stain films made from the same blood at different times, e.g. 5, 7,10,12 minutes. Compare results with a stained control blood film.

Check the pH of newly prepared buffered water and re-check it at weekly intervals. The pH of buffered water used to dilute the stain must be correct as it mainly affects the staining reactions. Maintain consistency in the staining procedure by exactly following an SOP. If the quality of staining changes,

always report this to the person in charge of the laboratory and ensure the fault is remedied. The staining procedure should be checked at the beginning of each week(81).

Part IV: SOP for blood film for hemoparasite examination

I. Clinical significance

Blood film preparation and examination is performed for detection and identification of different blood parasites for the diagnosis of blood associated parasitic infections.

II. Principle

Blood is collected, smeared on a glass slide and stained to investigate the presence of a blood parasite like Plasmodium, Babesia, Trypanosoma species, Leishmania donovani and microfilaria. The simplest method for detecting malaria and babesia continues to be the blood film. The thick film provides the greatest sensitivity and should be performed on all malaria requests. Thin films have a lower sensitivity and are primarily used for species identification.

III. Specimen required

Whole blood

IV. Materials and equipments required

Frosted slide	Pencil
Timer	Alcohol
Microscope	Cotton wool
Wash bottle	Staining troughs
Slide forceps	Measuring cylinders, 10, 50 and 100 ml
Giemsa Stain stock solution	Slide racks
Beakers, 50 and 250 ml	Absolute Methanol
Buffered water, pH 7.2, or distilled water	Waste and sharps disposal containers

V. Blood collection (thick and thin films on same slide)

The most suitable time for collection is at the height of an episode of fever, when the parasites are most numerous in the blood. Blood specimens should always be collected before anti-malarial drugs are given (See Annex III Part I for blood collection process).

1. Using a completely clean grease-free microscope slide, add a small drop of blood to the centre of the slide & a larger drop about 15 mm to the right.
2. Immediately spread the thin film using a smooth edged slide spreader. Blood from anemic patients needs spreading more quickly with the spreader held at a steeper angle.
3. Without delay, spread the large drop of blood to make the thick smear. Cover evenly an area about 15 x15 mm. It should just be possible to see (but not read) newsprint through the film.
4. Using a black lead pencil, label the slide with the date and patient's name and number. If a slide that has no a frosted end is used, write the information neatly on the top of the thin film (after it has dried).

5. Allow the blood to air-dry with the slide in a horizontal position and in a safe place protected from flies, dust and extreme heat.

VI. Staining

Principles of Giemsa stain method

During staining of the blood film, the Hb in the erythrocytes dissolves (dehemoglobinization takes place), and is removed by the water in the staining solution. Giemsa stain is an alcohol-based Romanowsky stain, which is a mixture of eosin and methylene blue. The chromatin and stippling of the parasite stain red or pink by the eosin, and the cytoplasm of the parasite stains blue by methylene blue.

Staining procedure

1. Fix the air-dried thin film by adding three drops of methanol, or by dipping it into a container of methanol for a 10-20 seconds.
2. Immediately before use, dilute the Giemsa stain as required: 10% solution by adding 45 ml of buffered water, pH 7.1-7.2 in a 50 ml cylinder, and 5 ml of Giemsa stain stock solution (to 50 ml mark) and mix gently.
3. Place the slides smears facing away from each other in a staining rack for immersion in a staining trough.
4. Thick blood films must be thoroughly dried and thin blood films must be fixed.
5. Pour the diluted stain into the staining trough and wait for 10 minutes.
6. Wash the stain from the staining container using clean water.
7. Wipe the back of each slide and place it in a draining rack for the preparation to air-dry.

VII. Microscopic examination and reporting

When the film is completely dry, apply drop of immersion oil to an area of the film that appears mauve coloured (usually around the edges) and examine the slide under microscope using 100X objective.

Thick blood films

In thick blood films, the background should be clean and free from debris, as the infected erythrocytes are lysed. The malaria parasites should have deep red chromatin and blue or pale purplish-blue cytoplasm. In thick films stained with Giemsa, the nuclei of leukocytes should be stained dark purple. Schüffner's dots may be seen around the malaria parasites. Thick blood films are used for estimating the parasite density.

Parasite density

The parasite density is the number of parasites counted in each microscope field. It usually varies according to the species. Two methods can be used to count malaria parasites in thick blood films: determination of the number of parasites per microliter (µl) of blood, and the plus system.

Determination of the number of parasites/µl of blood is accomplished by counting the number of parasites in relation to a standard number of leukocytes/µl (8000). Requires observation of at least 100 fields while you count 200 WBCs. Number of asexual stage of parasites and WBCs should be counted in each field until the number of WBCs reach 200.

Initially, the blood film is examined for the presence of parasite species and their stages of development. Using two hand tally counters, one for counting leukocytes and the other for parasites, calculate the parasite load using the following formula.

$$\text{Parasites/}\mu\text{L} = \frac{\text{Parasites counted}}{\text{WBCs counted}} \times \text{WBCs / }\mu\text{L}$$

Equation 3: Formula for calculating the parasite load from a blood film

A simpler method of counting parasites in thick blood films is to use the plus system. This system is less satisfactory, however, and should only be used when it is difficult to carry out counting parasites/µl of blood. In this system, a code of between one and four plus signs is used:

- 1+= 1-10 parasites per 100 thick film fields
- 2+ = 11-100 parasites per 100 thick film fields
- 3+ = 1-10 parasites per single thick film field
- 4+ = more than 10 parasites per single thick film field

Remember: For proper identification and reliable parasite counting, use clean slides, and well-made and well-stained thick films.

Note: Patients with very high parasite densities (over 10 parasites per thick film field) require **urgent treatment**. Therefore, if you find a high parasite density, state the result clearly in your report and send it immediately to the patient's physician.

Reporting results

If the result of the examination of the stained blood films is positive, specify:

- The species of parasite found
- The stage of development of the parasite
- The parasite density

Blood films containing P. *ovale* and P. *vivax* may contain few parasites, and therefore take more time to examine under the microscope. However, it is necessary to differentiate the two species, since they may re-appear in the blood without re-infection. Patients infected with P. *ovale* or P. *vivax* require additional treatment to eradicate the liver stages of these parasites.

A patient can harbour more than one species of malaria parasite at the same time (e.g. P. *falciparum* and P. *malariae* or P. *ovale* and P. *vivax*).

Thin blood films

In thin blood films, the infected erythrocytes may remain unchanged or have a different colour or shape, or may contain pink ("Schüffner's") or red ("James") dots. Thin films are performed for:

- Species identification
- Quantification e.g. percentage of infected red cell
- Useful in high parasitemia

Proportion of parasitized erythrocytes (thin film)

$$\% \text{ parasitemia} = \frac{\#\text{ of parasitized RBCs}}{\text{Total RBCs counted in 25 fields}} \times 100$$

Equation 4: Formula for calculating proportion of parasitized erythrocytes from a blood film

The number of parasitized erythrocytes (asexual forms) present in 25 microscopic fields is counted and divided by total number of RBCs present in these fields (about 5000), and multiplied by 100.

If the result is negative, report as "**no hemoparasite seen**"(80).

Part V: SOP for stool examination

A. Direct wet-mount technique

I. Clinical significance

Direct wet mount technique is used for identification of ova, larval, and adult stages of a parasite for diagnosis of different intestinal parasitic infestations.

II. Principle

Wet preparation on fresh unpreserved stool specimen is performed and examined as soon as possible (within 30 minutes of passage). The value of wet preparations lies in the fact that certain protozoa trophozoites retain their motility which may aid in their identification.

I. Specimen required

Fresh stool (within 30 minutes of passage)

II. Materials and equipments required

Glass microscope slide	Cover slide (22 x 22 mm)
Wooden applicator sticks	Microscope
0.85% Normal saline	Pasteur pipettes
Sharps disposal container	

III. Procedure

1. Add one drop of 0.85% normal saline on slide
2. Using a wooden applicator stick put a small portion of stool on the slide, (if the stool is formed take the piece from inside & the surface of the sample or if stool is watery take a drop from any part)
3. Mix the sample with the drop of normal saline on the slide and cover it with 22 X 22 mm cover glass. The sample should be spread thinly enough that newsprint can barely be read when the slide is placed on top of the text
4. Put the slide on microscope and observe with the 10X objective, and then switch to 40X objective for more detailed study of any suspect eggs or protozoa.

Note; for reporting look the formol-ether concentration technique reporting procedure

B. Formol-ether concentration technique

I. Clinical significance

Fecal concentration is a routine part of the ova and parasite examination, which allows the detection of small numbers of organisms that may be missed by using a direct wet smear for diagnosis of intestinal parasitic infestations.

II. Principle

In this method feces are emulsified in formol water, the suspension is strained to remove large fecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the fecal debris is separated in a layer between the ether and the formol water. Fecal fat is dissolved in the ether.

III. Specimen required

Stool preserved in SAF (Sodium acetate acetic-acid formalin)

IV. Materials and equipments required

Glass microscope slide — Cover slide (22 x 40 mm)
Wooden applicator — Microscope
Formol water, 10% v/v — Diethyl ether or ethyl acetate
Sieve (strain) with small holes, preferably 400-450 μm in size

V. Procedure

1. Using a rod or stick, emulsify an estimated 1 g (pea size) of representative stool sample in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.
2. Add further 3-4 ml of 10% formol water, cap the bottle and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical tube and add 3-4 ml of diethyl ether or ethyl acetate
   Caution: Ether and ethyl acetate are flammable, therefore use well away from an open flame.
5. Stopper the tube and mix for 1 minute.
6. With a tissue or a piece of cloth wrapped around the top of the tube, loosen the stopper.
7. Centrifuge immediately at 3000 rpm for 1 minute.
8. Using a stick or bulb of plastic pipette, loosen layer of fecal debris from the side of the tube & invert the tube to discard ether, fecal debris and formol water. The sediment will remain.
9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to re-suspend and mix the sediment. Transfer the sediment to the slide, and cover with cover glass. To assist the identification of cysts run a drop of iodine under the cover glass.
10. Examine the preparation microscopically using 10X objective with the condenser closed sufficiently to give good contrast. Use 40X objectives to examine small cysts and eggs.

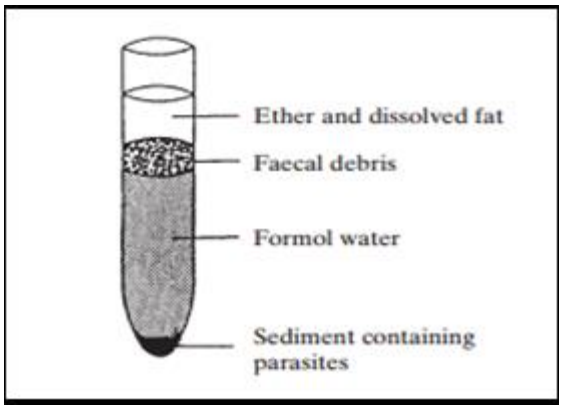

Figure 5: Appearance of contents by formol-ether concentration technique, after centrifugation (79).

VI. Reporting

Use Bench Aids in the diagnosis of Intestinal Parasites

If there is any parasite seen, report its **species and stage** of development

If there is no any parasite observed, report as "**No ova/parasite seen**" (79).

Part VI: SOP for MUAC measurement

1. Locate the tip of left shoulder with your finger-tips and bend the elbow to make a right angle
2. Place the MUAC with zero at the shoulder and pull it straight down to the elbow
3. Read the number at the tip of the elbow to the nearest centimeter and divide this number by two to estimate the midpoint
4. Mark the midpoint with a pen on the arm
5. Straighten the arm and wrap the tape around the arm at midpoint, making sure the numbers are right side up and the tape is flat around the skin.
6. Inspect the tension of the tape on the arm so that it has the proper tension. Proper tension is that the tape is not too tight or too loose.
7. When the tape is in the correct position on the arm with the correct tension, read and call out the measurement to the nearest cm(77).

ANNEX IV: Laboratory request and report format

JIMMA UNIVERSITY COLLEGE OF HEALTH SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY

Hematological and Parasitological investigation of pregnant women in South Sudanese refugees attending ANC Clinic of Pugnido ARRA Health Center, Gambela, Western Ethiopia (April 15 to June 30/2015).

1. Personal data

1.1. Code no._____

1.2. Age_____

1.3. Address _____

1.4. Occupation _____

1.5. Date of sample collection _____

2. ***Laboratory data***

2.1. Parasitological data

2.1.1. Stool examination

A. Physical examination

Consistency of stool

Formed ☐ Watery ☐ Semi-formed ☐

Appearance/Color

Normal ☐ Blood stained ☐ Pale yellow ☐ Mucoid ☐

B. Microscopic examination

Direct wet-mount technique

Species and stage of parasite seen _____

No ova/parasite ☐

Concentration technique

Species and stage of parasite seen _____

No ova/parasite ☐

2.1. 2. Blood film examination result

A. Species and stage of hemoparasite seen _____

B. Load of hemoparasite seen _____

2.2. Complete blood count result

Parameters	**WBC**	**Lymphocyte**	**Neutrophils**	**Mid**	**RBC**	**Hb**	**Hct**	**MCV**	**MCH**	**MCHC**	**RDW**	**Platelets**
Result												

2.3. Blood film morphological examination result

Size_____

Shape_____

Hemoglobinization_____

Inclusion_____

Name of investigator: _____

Signature: _____ Date: _____

Note;

This format is developed for this particular research purpose, and should only be applied for this study to record laboratory investigation results of pregnant women involved in this study. The laboratory personnel who analysed the specimen should complete it appropriately.

ANNEX V: Declaration Sheet

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

Principal investigator

Mr. Aklilu Alemayehu (BSc., MSc. student at Jimma University)

Signature _____ Date of submission_____

This thesis has been submitted for examination with my approval as University advisor.

Advisors

1. Dr. Tilahun Yemane (MD, MSc.; Lecturer at Jimma University)

   Signature _____ Date_____

2. Mr. Lealem Gedefaw (MSc., Assist prof; Lecturer at Jimma University)

   Signature_____ Date_____

3. Mr. Yaregal Asres (BSc., MSc.; Lecturer at Jimma University)

   Signature_____ Date_____

Examiners:

External examiner: Dr. Demisew Amenu (MD, MSc.; Lecturer at Jimma University)

Signature_____ Date_____

Internal examiner:

Mr. Girum Tesfaye (BSc., MSc.; Lecturer at Jimma University)

Signature_____ Date_____