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Magnitude, Severity and Associated Factors of Anemia among under-five Children attending Hawassa University Teaching and Referral Hospital and Adare Hospital in Hawassa, Southern Ethiopia, 2016

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This is to certify that the thesis prepared by Yeshimebet G/Selassie, entitled:

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii
LIST OF TABLES vi
LIST OF FIGURES vii
ABBREVIATIONS viii
OPERATIONAL DEFINITIONS ix
Abstract x
1. INTRODUCTION 1
1.1 Background 1
1.2 Statement of the problem 3
1.3 Rationale of the study 5
2. LITRATURE REVIEW 6
2.1 Magnitude of Anemia 6
2.2 Contributing factors of anemia 9
2.3 Laboratory Diagnosis of anemia 10
3. OBJECTIVES 11
3.1 General Objective 11
3.2 Specific Objectives 11
4. HYPOTESIS 11
5. MATERIAL AND METHODS 12
5.1 Study area 12
5.2 Study period 12
5.3 Study design 12
5.4 Population 12
5.4.1 Source population 12
5.4.2 Study population 12
5.5 Inclusion and Exclusion criteria 13
5.5.1 Inclusion criteria 13
5.5.2 Exclusion criteria 13
5.6 Study variables 13
5.6.1 Dependent variables 13

5.6.2 Independent variables 13
5.7 Measurement and data collection 14
5.7.1 Sample size and sampling technique 14
5.7.2 Data collection 14
5.7.3 Hematological analysis 15
5.8 Stool specimen collection and processing 16
5.8.1 Direct smear microscopy 17
5.8.2 Formol-ether concentration 17
5.9 Data Quality Assurance 17
5.9.1 Pre analytical 17
5.9.2 Analytical 17
5.9.3 Post analytical 18
5.10 Data analysis 18
5.11 Ethical considerations 18
5.12 Dissemination of result 19
6. RESULTS 20
6.1 Characteristics of Study Participants. 20
6.2 Parents /guardians' Characteristics 20
6.3 Magnitude of anemia in under five children 22
6.3.1 Severity of anemia in under five children 23
6.4 Anemia and associated risk factors 25
6.5. Associated risk factors of severe anemia 30
7. DISSCUSION 33
8. STRENGTH AND LIMMITATION OF THE STUDY 38
8.1. Strength of the study 38
8.2. Limitation of the study 38
9. CONCLUSION AND RECOMMENDATIONS 39
9.1. Conclusion 39
9.2. Recommendations 39
10. REFERENCES 40
11. ANNEXES 45
Annex I: Questionnaire 45
Questionnaire /Amharic version/ 46

Annex II: Information sheet for study participants 48
Annex III: Consent form for study participants..... 51
Annex IV: Laboratory report form 53
Annex V: Laboratory techniques 54
Annex VI: Procedure and reagents of Cell-Dyn 1800 Hematology Analyzer 56
Annex VII: Procedure for cell Dyn 1800 hematology analyzer 57
Declaration 60

LIST OF TABLES

PAGE

Table 1. Socio-demographic characteristics of children aged 6-59 months and their Parents/guardians attending Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016----------21

Table 2. Distribution of hemoglobin level among male and female under 5 children at Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016. ----------24

Table 3. Level of anemia among under 5 children categorized by age at Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016 ---------- ------24

Table 4. Frequency of intestinal parasites among children under5 children in Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016 ----------25

Table 5. Factors Associated with anemia among children aged 6-59 months in Hawassa University Teaching and Referral hospital and Adare Hospitals, South Ethiopia, 2016 ----------27

Table 6. Multivariate logistic regressions of selected variables associated with anemia among under five children in Hawassa University Teaching and Referral hospital and Adare hospitals South Ethiopia, 2016. ---------- 30

Table 7. Socio- demographic Characteristics and clinical findings associated with severity of anemia among under five children at Hawassa University Teaching and Referral hospital and Adare hospitals, Hawassa South Ethiopia, 2016. ----------31

LIST OF FIGURES

PAGE

Figure 1. Workflow----------15

Figure 2. Frequency of anemia by sex among under five children in Hawassa University Teaching and Referral hospital and Adare Hospitals Hawassa, South Ethiopia, 2016 ----------22

Figure 3. Frequency of anemia by age categories among under five children in Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016.----------23

ABBREVIATIONS

AOR _____Adjusted odds ratio

CBC _____Complete blood count

CI _____Confidence interval

COR _____Crude odds ratio

EDTA _____ Ethylene Diamine Tetra Acetic acid

EDHS _____ Ethiopian Demographic and Health Survey

fL _____ Femtoliter

g/ dl _____ Gram per deciliter

Hct _____ Hematocrit

Hgb _____ Hemoglobin

IP _____ Intestinal Parasites

MCH _____ Mean Corpuscular Hemoglobin

MCHC_____ Mean Corpuscular Hemoglobin Concentration

MCV _____ Mean Corpuscular Volume

NCNC_____ Normocytic Normochromic

OPD _____ Outpatient department

RBC _____ Red Blood Cells

RDW_____ Red cell distribution width

SNNPR_____ Southern Nations Nationalities and People Region

SOPs _____ Standard operating procedures

SPSS _____ Statistical Package for Social Sciences

SD_____ Standard deviation

WHO _____ World Health Organization

µl _____ Micro liter

OPERATIONAL DEFINITIONS

Anemia in children: defined based on WHO classification as hemoglobin level of less than 11 g/dl.

Mild anemia: Hgb level of 10 to 10.9 g/dl

Moderate anemia: Hgb level of 7 to 9.9 g/dl.

Severe anemia: Hgb level of less than 7 g/dl.

Abstract

Background: Anemia is a widespread public health problem associated with increased risk of morbidity and mortality. Infants, under 5-year-old children and pregnant women have greater susceptibility to anemia. The magnitude and associated risk factors for anemia vary in different settings.

Objective: To determine the magnitude, severity and associated factors of anemia among under five children attending at Hawassa University Teaching and Referral hospital and Adare hospital, Southern Ethiopia.

Methods: A hospital based cross-sectional study was conducted from December 2015 to June 2016 at Hawassa University Teaching and Referral hospital and Adare hospitals in Hawassa, Southern Ethiopia. A total of 422 under five children were included applying convenient sampling method. Socio demographic data and other predisposing factors were collected by using interviewer administered structured questionnaire. Venous blood sample were collected from each child and analyzed for hemoglobin determination using cell-Dyn 1800 automated analyzer. Stained thin and thick smears were prepared for malaria parasites detection. Stool samples were collected and processed using direct wet mount and formol-ether concentration method to detect intestinal parasites. Data was entered and analyzed using SPSS version 20 statistical packages. Binary and multiple logistic regressions were computed to assess association between variables. P-value less than 0.05 was taken as statistically significant.

Result: Among the 422 study participants, 235(55.7%) were males and 187(44.3%) were females. The mean hemoglobin level was 10.59 g/dl and about 176(41.7%) of children were anemic. Anemia was of mild, moderate and severe type in 6.6%, 19% and 16.1% of the children, respectively. Being in the age group 6–23 months [AOR = 2.04: 95% CI (1.13, 3.69)], and having mothers with no formal education [AOR = 1.73: 95%CI (0.99, 3.02)] were identified as associated factors for anemia.

Conclusion: The magnitude of anemia was remarkably high being predominantly of moderate and severe type. Children between 6 to 23 months and those who had mothers with no formal education were more likely to be anemic. Thus, interventions like health education for mothers/care givers should be strengthened.

Key words: Anemia, Under five children

1. INTRODUCTION

1.1 **Background**

World Health Organization (WHO) defines anemia as a decrease in the concentration of circulating red blood cells or in the hemoglobin (Hgb) concentration and a related impaired capacity to transport oxygen. It is also defined as hemoglobin level below 11mg/dl for children 6-59 months (1).

Anemia is classified as microcytic, normocytic, or macrocytic, based on the mean corpuscular volume (2). It can also be classified based on haemoglobinization of red cells as Normochromic, describing normal staining of red cells as seen when haemoglobinization is adequate, and hypochromic describing pale staining of red cells, as seen when haemoglobinization is inadequate. Hypochromic cells show an increased area of central pallor (3).

Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children which is 47.4%. WHO regional estimates generated for preschool-age children and pregnant and non-pregnant women indicate that the highest proportion of individuals affected are in Africa (47.5–67.6%), while the greatest number are in South-East Asia where 315 million individuals are affected (1).

WHO showed that 818 million children under the age of five and women are affected by anemia, mainly in developing countries. About one million of them die every year (1).

In the developing world, 42% of children less than five years of age and 53% of children 5–14 years of age are anemic (4). About 67.6% of under five children in Africa are suffering from anemia indicating anemia as a sever health problem. In Eastern Africa, it is estimated that three quarters of under five children suffer from anemia (1). Studies in east Africa have shown that *P. falciparum* malaria and iron deficiency account for much of the anemia seen in young children (6).

In Ethiopia, according to the demographic and health survey findings in 2011, more than four out of ten under-five children (44%) were anemic. From these, about 21% of children were mildly anemic, 20% were moderately anemic, and 3% were severely anemic (7).

The etiology of anemia involves the interaction between multiple factors including nutritional deficiencies, genetic red blood cell disorders, and infectious diseases, particularly malaria and hookworm infections. Human immunodeficiency virus/acquired immunodeficiency syndrome should also increasingly be considered as a direct and indirect contributor to anemia in young age group (9, 10, 12).

Like other developing countries, the prevalence of intestinal parasites is widely spread in Ethiopia that can cause anemia in under-five children. Among the common intestinal protozoan parasites Giardia, Cryptosporidium, and helminthes such as Ascaris are widely distributed, where there is overcrowding, poor environmental sanitation, limited economic resources and poor personal hygienic practice which are predisposing factors. Most of the intestinal parasites are more sever in children than adults, which is associated with malnutrition, growth retardation, and poor care for children. The case is worth in under five year old children because of poor maternal hygiene, play habitats of children in the house in close proximity to one another that create an appropriate condition for the transmission and spread of the disease (9).

A consequence of anemia, may lead to impaired cognitive function, growth and psychomotor development, especially in children. Infants, under 5-year-old children and pregnant women have greater susceptibility to anemia because of their increased iron requirements due to rapid body growth and expansion of red blood cells (5, 8, 12).

The frequency of anemia in a population may be the most important single indicator of the overall health status of a population regarding malaria, geo-helminthes infection and mal- or Under nutrition (12).

1.2 Statement of the problem

Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality (13). It is considered to be an important contributing factor to the global burden of disease. Affecting both developed and developing countries, it has an impact not only on human health and productivity but in the process it affects the socio-economic development of a nation (14, 15). WHO has developed a classification system to facilitate international comparisons of anemia as a public health problem, the problem is considered severe if anemia prevalence is ≥40%, moderate from 20% to 39.9%, and mild from 5% to 19.9% (15).

High prevalence of anemia and its consequences for children's health, and especially for their growth and development, have made anemia an important public health problem, given the difficulty in implementing effective measures for controlling it (16).

Anemia can occur at any time and at all stages of the life cycle but young children and pregnant women are the most at risk segment of the community (17). In East Africa approximately 75 % of under-five children have anemia with the prevalence's ranging between 44 and 76 % (18).

Between 1990 and 2012, mortality in children under 5 years of age declined by 47%, from an estimated rate of 90 deaths per 1000 live births to 48 deaths per 1000 live births. The risk of a child dying before their fifth birthday is still highest in the WHO African Region (95 per 1000 live births)– eight times higher than that in the WHO European Region (12 per 1000 live births) (19).

The risk factors for anemia vary in different settings; they include having intestinal parasites and malaria (20). In developing countries, insufficient dietary iron is considered the primary cause of anemia in children (21). Parasitic infection has been identified as one of the main cause of poor nutritional status. This has impact on iron status particularly hemoglobin level (22).

Anemia in pre-school children has a negative effect on cognition, motor development and growth, academic performance, immunity, and susceptibility to infections (14). Despite these, detailed investigation of anemia in under five children is limited in Ethiopia and existing data revealed variable magnitude..3

Therefore, this study is designed to assess the magnitude of anemia and its associated factors among under 5 children attending Hawassa University Teaching and Referral hospital and Adare hospital in Hawassa, Southern Ethiopia.

1.3 Rationale of the study

Anemia is a major problem among young children (aged 6-59 months old) mainly due to malaria, dietary deficiencies and parasitic infections.

The findings of this study provide evidence for policy formulation, problem prioritization and resource allocation to set prevention programs, update the treatment protocols for proper management, follow up and care of children with anemia. Thus, both clinicians managing patients, hospital administrators as well as policy makers could benefit from the information.

2. LITRATURE REVIEW

2.1 Magnitude of Anemia

Globally an estimated 293.1 million (about 43%) of children under five years are anemic of which 28.5% are residing in Sub Saharan Africa. It is considered to be a major public health problem with prevalence of 67%, equivalent to 83.5 million children in Sub Saharan Africa (20).

The report by Leite *et al* (2013) from the first national survey of indigenous people's health and nutrition conducted in Brazil children < 5 indicated that the overall prevalence of anemia was 51.2 % (23). In another study conducted by I, Malaquias Batista Filho *et.al*., (2013) in North eastern Brazil, in children aged 6-59 months the prevalence of anemia was 32.8% (16) .

A study conducted at public daycare centers in the city of Recife, Brazil by Vieira *et al* (2007) indicated that the prevalence of anemia (hemoglobin < 11.0 g/dL) was found to be 55.6% (24). Similarly based on the study conducted by Ferreira (2011) at children's hospital in Recife, Brazil the prevalence of anemia was 56.6% (10).

In a study by Siegel *et al* (2006) which was done in the community to describe the distribution of hemoglobin and prevalence of anemia in Nepali children living in the Terai region, the prevalence of anemia was found to be 58% (25). According to the study conducted by B. Alzain (2012) in North Gaza community, the prevalence of anemia was 65.3 % having hemoglobin levels lower than 11g/dl (13). Another community based cross-sectional study that was conducted by Rafiq*, et.al* (2015 in Gaza Strip- Palestinian among preschool children, revealed an overall anemia prevalence of 59.7%. Of which 46.5% and 13.5% had mild and moderate anemia, respectively (26).

A study conducted by UGC-CSSEIP Centre (2014) in two remote villages of India, on preschool children reported that the prevalence of anemia was 62% among whom, 48% of girls and 56% of boys were severely affected; while 47 % of girls and 41% of the boys were modestly affected and 10% of the boys and 28% girls observed mildly affected (27). The hospital based study conducted by Mk, Uddin *et al*., (2010) to determine the prevalence of

anemia in children of 6 to 59 months old in Narayanganj, Bangladesh ,on the other hand , revealed that the prevalence of anemia was 40% (28).

According to a cross-sectional study conducted by Tazhibayev, *et al*., (2014) on children aged 6-59 months from Aktobe, Pavlodar and North Kazakhstan counties, the overall respective prevalence of anemia was 48.6%, 44.9% and 40.0%. The prevalence of moderate and severe anemia was 17.9% and 1.6% in Aktobe, 20.3% and 0% in Pavlodar and 15.2% and 0.8% in North Kazakhstan counties (29).

On the other hand, a study that was done by Gao, *et al*., (2013) among children under 36 months old in rural community western China, showed that the prevalence of mild, moderate and severe anemia to be 27.4%, 21.9% and 3.2%, respectively (30).

Another community based study that was conducted in Burma by Zhao*et al*., (2012), to determine the prevalence of anemia and risk factors associated with it on children 6 to 36 months age , revealed 72.6% prevalence, with 40.0% having severe anemia (11).

The study conducted by Ayoya *et al*., (2013) in Haiti among children 6-59 months revealed that the prevalence of anemia was 38.8% with 23.9% mild, 14.7% moderate, and 0.2% having severe anemia (31).

Muoneke,*et al*.,(2012) determined the risk factors associated with poor outcome among under-five children with severe anemia in sub Saharan Africa. Accordingly, out of 1,450 patients admitted during the period of their study 140 had severe anemia with a prevalence rate of 9.7% (32).

A community based study conducted by G.A.Onyemaobi *et al*.,(2011) on children aged 6-60 months in Imo State, Nigeria revealed a prevalence rate of anemia of 70.5% , among these 38.0% had mild anemia, 31.8% were moderately anemic and 0.8% were severely anemic (33). According to a house hold study conducted by Semedo *et al*., (2013) to estimate the prevalence of anemia and associated factors in children aged 6-59 months in Cape Verde, West Africa, the prevalence of anemia was 51.8% (21).

A cross sectional hospital based study conducted in Kassala, Eastern Sudan by Mahmoud *et al*., (2014) indicated that 86% of all children were anemic, among these 64 % of them were severely anemic (34).

In a population-based, cross-sectional survey on children 6–35 months of age in western Kenya by Foote *et al*., (2013) the prevalence of Anemia was 71.8% and severe anemia was recorded in 8.4% of the participating children (35). Whereas another study conducted by Ngesa and Mwambi (2014) across Kenya, in the age of 6 months to 14 years, documented a prevalence rate of 28.8% (5).

A hospital-based, cross-sectional study conducted amongst under-five years old children hospitalized at Bugando Medical cener in Tanzania, by Simbauranga *et.al* (2014), revealed an overall anemia prevalence of 77.2 %. By severity mild, moderate and severe anemia was noted in 16.5%, 33% and 27.7 % of the children, respectively (20).

Unlike the hospital based study, the study that aimed to determine the prevalence of anemia and parasitic infections among under 5 years old children of northern Tanzania by Nyaruhucha *et al* indicated that 47.6% of children were anemic. Of them 20.8% were mildly anemic, 21.6% moderately anemic and 5.2% severely anemic (22).

According to community based study by Ewusie *et al*., (2014), the prevalence of anemia in Ghanaian children under the age of five was 78.4% where 7.8% of them had severe anemia, 48.0% moderate anemia and 22.6% had mild anemia (36).

Studies carried out in Ethiopia also revealed variable rates of anemia in under -fives. For example according to a study conducted by Gebreegziabiher *et al* (2014) in Kilte Awulaelo Woreda, northern Ethiopia, the prevalence of anemia among children aged 6–59 months was 37.3% (37). Whereas, an institution based cross-sectional study carried out on younger Children Aged 6–23 months attending growth monitoring at Tsitsika Health Center in Wag-Himra Zone, Northeast Ethiopia, by Woldie *et al* (2015) demonstrated an overall anemia prevalence of 66.6% (17).

Another study conducted by Habte, *et al* (2013) in children between the ages of 6-59 months to identify the risk factors associated with childhood anemia in Ethiopia , revealed that 50.3% were anemic (38).

On the other hand a study drawn from the third Ethiopian Demographic and Health Survey - 2011 (EDHS), revealed among children between the ages of 6-59 months (44%) were found

to be anemic. Among them mild, moderate, severe anemia was recorded in 21%, 20%, 3% , respectively (7).

Taken together, the studies reviewed above reported variable prevalence rates in some cases as high as more than 80% (34) in under-five children both in the community as well as in those studied in health institutions.

2.2 Contributing factors of anemia

The determinants of anemia look similar with little variation according to geographical region. Some studies showed that anemia was positively associated with malaria, malnutrition, nutritional deficiencies, HIV infection and low socioeconomic status. The study conducted to determine the risk factors associated with poor outcome among under-five children with severe anemia in sub Saharan Africa, identified Malaria as the most common cause of severe anemia with a prevalence rate of 64.3 % (32).

Globally, an estimated 3.4 billion people are at risk of malaria. WHO estimates that 207 million cases of malaria and 627 000 deaths occurred globally in 2012. Most cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) were in children under 5 years of age (39).

A study conducted by Foote, *et al*., (2013) in western Kenya, among factors commonly thought to be associated with anemia including malaria parasitemia the prevalence of malaria was found to be 32.5 % (35)

The study conducted to determine the prevalence and risk factors of malaria among children below five years of age in southern highland Rwanda indicated that among children infected by malaria anemia was observed in 82% (40).

A cross sectional survey carried out in a tertiary care hospital in Karachi indicated that the proportion of children who had stool positive test result was 68.8% and among them 44.6% were found to have mild anemia (41)

Study conducted by Sudhakar *et.al*., (2010) around Gilgel Gibe Hydroelectric dam South-West Ethiopia revealed that the prevalence of anemia was 32.4% and one tenth of the children had malaria parasite (42).

In Ethiopia, intestinal parasitic infections are the major causes of mortality and morbidity causing a series of public health problems such as malnutrition, anemia, and growth retardation as well as higher susceptibility to other infections (43).

A cross-sectional study which was conducted in Children under 5 years of age, by Mulatu.G *et al*., (2015) at Adare Hospital and Millennium Health Centre, located in Hawassa, South Ethiopia indicated that the overall prevalence of intestinal parasites was 26.6 % (44).

2.3 Laboratory Diagnosis of anemia

Hemoglobin and hematocrit measurements can be used together or separately to track and assess the prevalence of anemia (45). These parameters may be affected by factors, such as methods and equipment used for its determination. Hemoglobin levels cannot be derived from the hematocrit value with an acceptable accuracy using the rule of dividing by 3, because the relationship between hemoglobin and hematocrit changes with age during the first years of life (20). Studies carried out in more than 10,000 pairs of examinations in children under 2 years of age concluded that hemoglobin usually detects higher rates of anemia than hematocrit, as they identified more false negatives using the hematocrit <33%. The suggestion of this study to increase the cut-off of hematocrit <33% to <35% is one more argument to better assess its prevalence (45). However, the most reliable indicator of anemia at the population level is blood hemoglobin concentration (46) and hence this parameter is utilized in the current study to define anemia.

3. OBJECTIVES

3.1 General Objective

To determine the magnitude, severity and associated factors of anemia among under five children attending at Hawassa University Teaching and Referral hospital and Adare hospital, Southern Ethiopia, December 2015 to June 2016.

3.2 Specific Objectives

- To determine the magnitude of anemia among under five children attending Hawassa University Teaching and Referral hospital and Adare hospital.
- To classify anemia based on severity, as mild, moderate and severe using the hemoglobin value.
- To determine associated factors of anemia among under five years' old anemic children attending Hawassa University Teaching and Referral hospital and Adare hospital.

4. HYPOTESIS

- The magnitude of anemia is in the same range of what has been reported by EDHS 2011

5. MATERIAL AND METHODS

5.1 Study area

The study was conducted at Hawassa University Teaching and Referral hospital and Adare hospital, located in the city of Hawassa, South Nations and Nationalities People Region (SNNPR). Hawassa city is found about 270 km south of Addis Ababa covering 157.2 km^2 areas. Since 1991, the city is serving as the capital of Sidama zone. Currently the city administration has 8 sub cities and 32 local administrations (kebeles).The capital of SNNPR is located in the 7^0 29" latitude and $80^0$29" longitudes at 1708 meter above sea level and has temperate climate.

The hospitals provide medical service to a catchment population of fifteen million and are sites for practical training to medicine and health science students.

5.2 Study period

The study was carried out from December 2015 to June 2016.

5.3 Study design

A cross-sectional hospital based study was conducted to determine the magnitude of anemia and associated factors among under 5 children at Hawassa city, Southern Ethiopia.

5.4 Population

5.4.1 Source population

All under 5 years old children visiting Hawassa University Teaching and Referral and Adare hospital's pediatric outpatient department (OPD) and emergency units for any medical service were considered as source population.

5.4.2 Study population

Children aged between 6-59 months attending Adare and Hawassa University Teaching and referral hospitals visiting pediatric OPD and emergency units during the study period, fulfilling the selection criteria were the study population.

5.5 Inclusion and Exclusion criteria

5.5.1 Inclusion criteria

All children aged 6 to 59 months who were visiting hospitals during the study period and whose parents consented to participate in the study.

5.5.2 Exclusion criteria

- Children who had bleeding disorder
- Children who have taken blood and blood products for the last 3 months
- Children who were taking iron and Vitamin-A supplement recently.
- Children who taken anthelmintic drug in the previous six months

5.6 Study variables

5.6.1 Dependent variables

- ✓ Presence of anemia
- ✓ Severity of anemia

5.6.2 Independent variables

- ✓ Age
- ✓ Sex
- ✓ Presence of malaria parasite
- ✓ Presence of intestinal parasites
- ✓ Mother's Age,
- ✓ Mother's Education
- ✓ Number of children less than 5 years
- ✓ Residence

5.7 Measurement and data collection

5.7.1 Sample size and sampling technique

The minimum sample size required for analysis was calculated using the 95% confidence interval with 5% marginal error.

Using the formula $n= z^2p (1-p) / d^2$

Where n= sample size, z = statistic for a level of confidence (z =1.96 at 95% CI), p = expected prevalence or proportion (p =0.50, d = precision (if 5%, d= 0.05), the sample size was 384. Allowing 10% for non-response, the final sample size was 422.

Sampling technique

- Convenient sampling technique was used to select under five year's children who fulfill inclusion criteria during the study period.

5.7.2 Data collection

Semi-structured questionnaire was used to collect socio-demographic data and risk factors. The tool was prepared after reviewing different related literatures inside and outside the country. The original questionnaire which was prepared in English language was translated into Amharic version and used by trained nurses for data collection. In addition, blood and stool samples for laboratory analyses were collected and analyzed by experienced laboratory professionals.

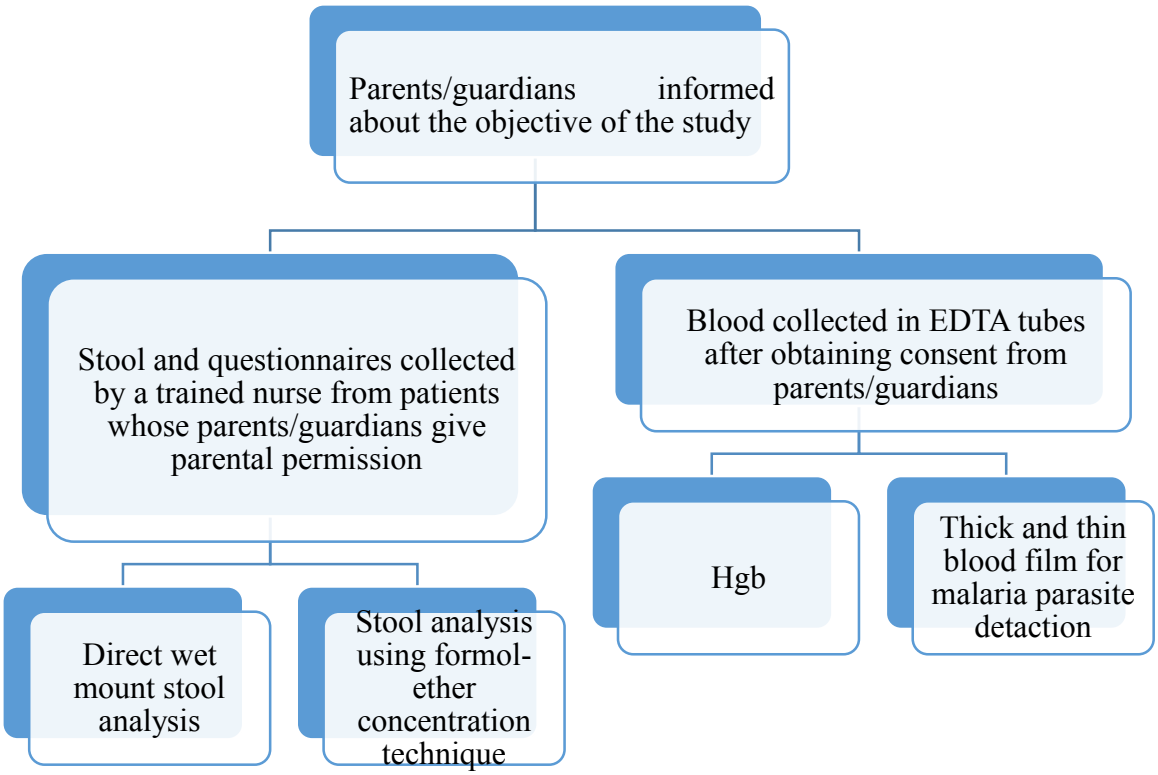

Figure 1. Workflow

5.7.3 Hematological analysis

5.7.3.1 Blood sample collection

About 3 ml venous blood samples were collected by strictly following standard operating procedure (SOPs). Experienced laboratory technicians collected the samples in tube containing ethylene diamine tetraacetatic acid (EDTA).

The blood collected from each child was processed for hemoglobin test. The Complete Blood Count (CBC) report from the hematology analyzer in the hospitals include; red blood cell (RBC), hemoglobin, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW); analyses were done as per the manufacturer's instruction. The Hawassa University teaching and referral hospital laboratory uses an automated cell counter CELL DYN 1800. Hematological analysis (Hgb determination) was performed in Hawassa University Teaching and referral hospital laboratory under suitable conditions.

5.7.3.2 Principle of Cell-Dyn 1800 Hematology Analyzer

Cell-Dyn 1800 hematology analyzer follows a method of counting and volumetric sizing based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid as it is drawn through a small aperture. It employs the non-conductive nature of blood cells.

The Cell-dyn 1800 hematology analyzer can handle up to 10,000 samples with the expanded data management system. It incorporates a 3-part differential and 18 parameter blood count. An optional bar code ready may be used to eliminate transcription data entry errors. Data may be output to both ink jet and dot-matrix printers. The Cell-Dyn 1800 uses only 3 reagents including cyanide-free differential lyse that reduces bio-hazards and reduces safety risks (47).

5.7.3.3 Classification of anemia

From the CBC result children with anemia as defined by Hgb level below 11mg/dl (WHO) criteria were selected.Anemia severity determined based on Hemoglobin value (15). Thick blood smears were also stained using Geimsa stain and examined for the presence of malaria parasites with 100X oil-emersion objective.

5.8 Stool specimen collection and processing

A fresh fecal specimen was collected using labeled, clean, dry, leak-proof container. The study participants (parents of the children) were oriented on how to provide stool specimen by a trained laboratory technician. Every individual was instructed to bring sufficient amount, 1 gm (pea size), of stool (48).

Those stool samples that were small in amount and those collected by containers other than they were provided with were rejected. Fecal specimens of improper collections were rejected based on rejection criteria.

Stool specimen was processed by wet mount and formol-ether concentration technique following WHO guideline (49) (Annex V) as briefly described below.

5.8.1 Direct smear microscopy

Direct smear was done by emulsifying about 2 mg of stool using physiological saline (0.85% NaCl solution) on a microscopic slide and was examined by 10x and 40x microscope objective (49) (Annex 4).

5.8.2 Formol-ether concentration

Stool samples were emulsified in formol water, the suspension was strained to remove large fecal particles, then ether was added, and the mixed suspension was centrifuged. The fecal debris was separated in a layer between the ether and the formol water. The supernatant was discarded and a small drop of sediment was placed on a microscopic slide and examined by 10x and 40x microscope objective (49) (Annex 4).

5.9 Data Quality Assurance

To assure the quality of data, training was given for data collectors and daily supervision was made during the data collection period.

- Quality assurance was maintained in pre analytical, analytical and post analytical stages.

5.9.1 Pre analytical

- Standard operating procedures (SOPs) and manufacturers' instruction was strictly followed for all laboratory activities.
- All laboratory reagents were checked for their expiry date.
- Samples were checked whether they are in the acceptable criteria like; hemolysis, clotting, volume and collection time.
- Microscopic slides and cover glasses were checked for cleanliness.
- Detail orientation was also given for clinical data and specimen collectors.

5.9.2 Analytical

- Three level whole blood controls (High, Normal, and Low) were run on the automated hematology analyzer and patients sample was analyzed when controls pass.
- The quality of the blood slides for malaria parasites was checked by two different experienced laboratory professionals.

5.9.3 Post analytical

- The Principal Investigator checked questionnaires daily for completeness and consistency of the responses.
- The test results were kept confidential.
- Each laboratory test result was recorded, reported and specimens were managed properly

5.10 Data analysis

All questionnaires were checked for completeness. Completed data were coded, entered, cleaned and analyzed using SPSS (Statistical Package for Social Sciences) version 20. Descriptive statistics (mean and standard deviation (SD) were used to summarize continuous variables and simple frequencies were computed to show the distribution of the socio-demographic and clinical characteristics of the patients. To assess associations between risk factors and anemia, bivariate and multivariate logistic regression analysis were used. Statistical significance was declared at P value < 0.05.

5.11 Ethical considerations

Ethical clearance was obtained from the ethical review board of Addis Ababa University College of Health Science, School of Allied Health Science, Department of Medical Laboratory Sciences and Institutional Review Board of the college of Medicine and Health Science of Hawassa University. In addition, letters of permission was written to all respected offices. Prior to data collection, written informed consent was obtained from each parents/caregiver of the study participants after explaining the purpose of the study. Confidentiality of the information was assured and privacy of the respondents was maintained. The study participants were exempted from paying any cost for the tests. Cost of any additional test was covered by the principal investigator. Parents or guardians were informed that the instruments and procedures used in the study do not cause any harm to the study participants and their right to withdraw from the study anytime. Positive results were communicated to the physician for further diagnosis and treatment.

5.12 Dissemination of result

Findings of the study will be presented to the College of Health Science, School of Allied Health Science Department of Medical Laboratory Sciences as partial fulfillment for the award of Degree of Master of science in clinical laboratory science (Hematology and Immunohematology). The dissemination will also goes to Hawassa University Teaching and Referral hospital, Southern Nations Nationalities and Peoples Region (SNNPR) Health Bureau. Attempts will be made to present the findings on various scientific conferences. In addition, an effort will be made to publish the finding of this study in a peer reviewed scientific journal.

6. RESULTS

6.1 Characteristics of Study Participants.

A total of 422 under five children participated in the study; of the participants, 235(55.7%) were males and 187(44.3%) females. The age of studied children ranged from 6-59 months with mean (±SD) age of 27.9 (±16.72) months. Regarding their age distribution, 174 (41.2%) were between 6-23months, 98 (23.2%) were between 24-35 months, 75(17.8%) were between 36-47 months and 75(17.8) were 48-59 months (Table 1).

6.2 Parents /guardians' Characteristics

Parents/guardians of the participating children were interviewed and their socio-demographic characteristics are summarized in Table 1. All of them were females and the age of respondents range from 18-40 years with mean (±SD) age of 28.8years (±4.99). Regarding the educational status 119 (28.2%) had attained secondary education and above, 112(26.5%) were at primary level and 191(45.3) had no formal education. Majority, 194 (36.5%), of the respondents were protestant followers while the least proportion 14 (3.3%) were catholic followers; 140(33.2%) of the respondent were housewives and 284(67.3%) living in urban area (Table 1).

Children who were on anti-parasitic treatments and whose parents did not consent to participate in the study had to be excluded from the study. However, none of the children were on anti-parasitic treatments prior to diagnosis and all parents/guardians consented to take part in the study.

Table 1. Socio-demographic characteristics of children aged 6-59 months and their Parents/guardians attending Hawassa University Teaching and Referral hospital and Adare Hospital, Hawassa, South Ethiopia, 2016

Parameters	Frequency	Percentage
Age of child (in months)		
6-23	174	41.2
24-35	98	23.2
36-47	75	17.8
48-59	75	17.8
Sex of child		
Male	235	55.7
Female	187	44.3
Parents/Guardians characteristics		
Residency		
Urban	284	67.3
Rural	138	32.7
Age group (in year)		
<30	241	57.1
>=30	181	42.9
Educational status		
No formal education	191	45.3
Primary	112	26.5
Secondary and above	119	28.2
Occupation		
Housewife	140	33.2
Employed	116	27.5
Small scale business	79	18.7
Farmer	80	19.0
Others	7	1.6
Religion		
Orthodox	137	32.5
Muslim	117	27.7
Protestant	154	36.5
Catholic	14	3.3

6.3 Magnitude of anemia in under five children

The overall prevalence of anemia as defined by hemoglobin levels lower than 11 g/dl was found to be 41.7% (176/422). Of them, 44.3% (78/176) were females and 55.7% (98/176) were males (Figure 2). The frequency of anemia declined with age; it was highest in the youngest age group of less than 2 years (48.9%) and lowest (34.7%) in the oldest age group (Figure 3).

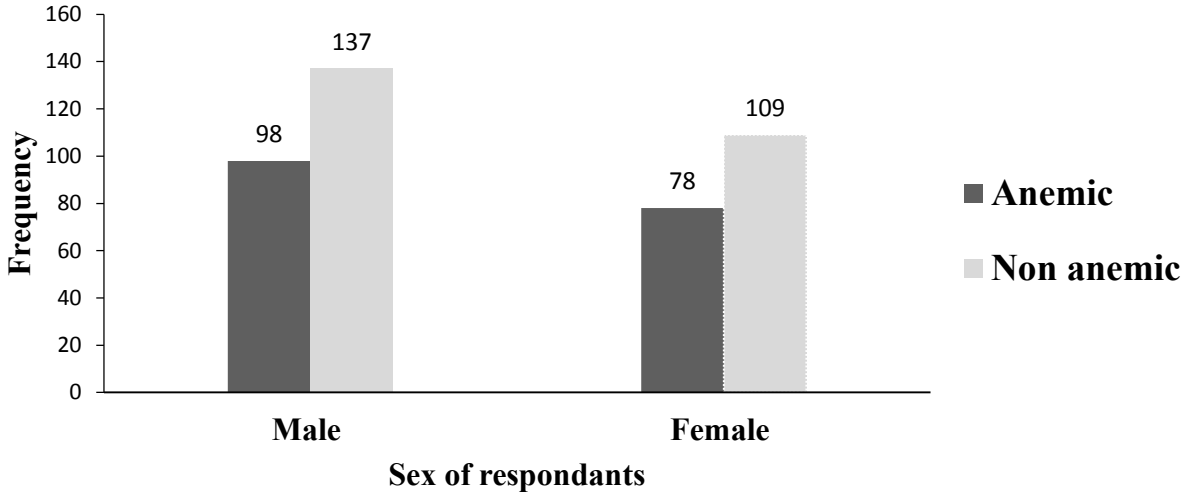

Figure 2. Frequency of anemia by sex among under five children in Hawassa University Teaching and Referral hospital and Adare Hospital Hawassa, South Ethiopia, 2016

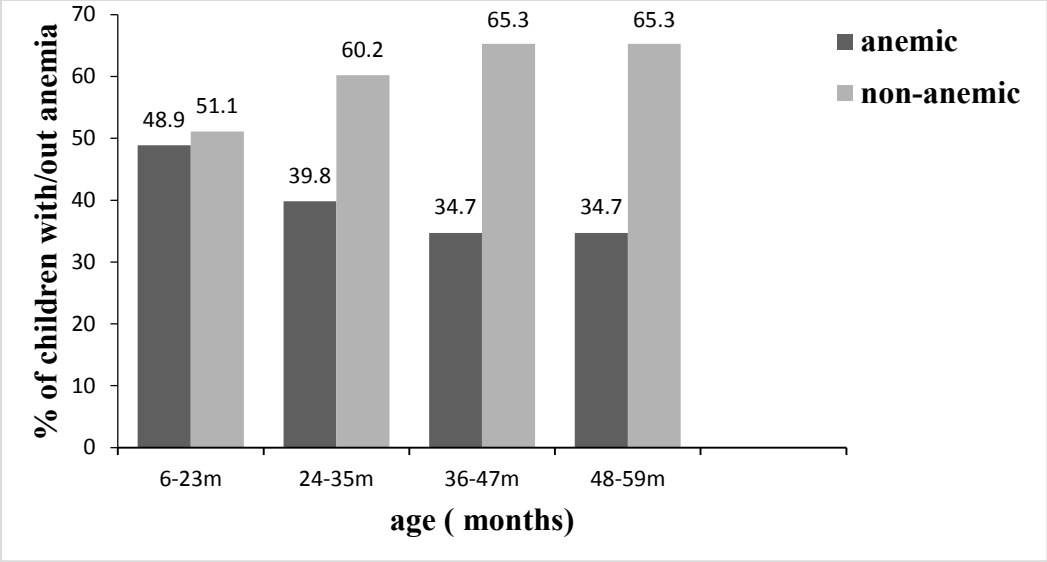

Figure 3. Frequency of anemia by age categories among under five children in Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016.

6.3.1 Severity of anemia in under five children

A child with hemoglobin levels lower than 11 g/dl was diagnosed as anemic based on the WHO standards. Accordingly, the mean hemoglobin level was about 10.59 g/dl (±3.04 SD) which ranged from 2.4 g/dl to 18.5g/dl. Based on degree of anemia 68 (16.1%) of them were found to be severely anemic, whereas 80 (19%) were moderately anemic and 28 (6.6%) were mildly anemic (Table 2). Younger children were more anemic and it gradually decreases as the child gets older. Anemia was of less severe type in those older children (48-59 months) as well (Table 3).

Table 2. Distribution of hemoglobin level among male and female under 5 children at Hawassa University Teaching and Referral hospital and Adare Hospital, Hawassa, South Ethiopia, 2016.

Hgb value	Male (%)	Female (%)	Total (%)
<7g/dl (severe)	40(17.0)	28(15.0)	68(16.1)
7-9.9g/dl (moderate)	40(17.0)	40(21.4)	80(19.0)
10-10.9g/dl (mild)	18(7.7)	10(5.3)	28(6.6)
≥11g/dl (non-anemic)	137(58.3)	109(58.3)	246(58.3)

Table 3. Level of anemia among under 5 children categorized by age at Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016

Age (months)	Non-anemic	Mild anemia	Moderate anemia	Severe anemia	Total
	N (%)	N (%)	N (%)	N (%)	N
6-23	89(51.2)	15(8.6)	40(23.0)	30(17.2)	174
24-35	59(60.2)	1(1.0)	16(16.3)	22(22.4)	98
36-47	49(65.3)	3(4.0)	14(18.7)	9(12.0)	75
48-59	49(65.3)	9(12.0)	10(13.3)	7(9.3)	75
Total					422

6.4 Anemia and associated risk factors

To determine the associated risk factors, anemia was denoted as dichotomous dependent variable where 1 was coded for those children who had anemia while 0 was coded non-anemic. So the odds ratio explained the chance of probability of suffering from anemia according to the predictor variables. The risk factors were described as follows.

Intestinal parasitosis among under five children

Among the risk factors, examination for intestinal and hemoparasites were carried out in all the 422 children. While none were positive for malaria parasites, this may be due to special prevention and control strategies by regional health bureau and health facilities.The overall result of intestinal parasitic etiologies among under five years children in Hawassa University Teaching and Referral hospital and Adare hospitals identified four types of parasites. All in all, 77 (18.2%) were infected with intestinal parasites. As shown in Table 4, *Ascaris lumbricoides* accounted the highest number which is 47(11.1%) followed by *E.histolyica* 18(4.3%), hookworm17 (4.0%) and *G.lambalia* 3 (0.7%). The prevalence rate was 44 (18.7%) for male and 33 (17.6%) for female. It was high among age group of 48-59 months (32%) compared to other age groups.

Table 4. Frequency of intestinal parasites among under5 children in Hawassa University Teaching and Referral hospital and Adare Hospitals, Hawassa, South Ethiopia, 2016

Species	**Positive**	**Negative**
	N (%)	N (%)
Ascaris lumbricoides	47(11.1)	375(88.9)
Hookworm	17(4.0)	405(96)
Entamoeba histolytica	18(4.3)	404(95.7)
Gardia lambalia	3(0.7)	419(99.3)

Bivariate and multivariate logistic regression analyses were done to look for association of socio-demographic and other maternal factors with child anemia.

In the bivariate logistic regression analysis, initially we have observed the association of each independent variable with the dependent variable as displayed in Table 5.

The variables that were statistically associated with anemia in this study were the child's age, residence, mother's education level, intestinal parasites and being infected specifically with *Ascaris lumbricoides*.

Age of child

Among the study participants, 41.2% were below 2 years of age. Age of the child was found to be significantly strongly associated with anemia [p=0.017]. Multivariate analysis showed that children aged 6–23 months were 2.04 (1.13, 3.69) times more likely to be anemic than those of 24–59 months (Table 6).

In our finding the frequency of anemia among 36-47 months and 48-59months was equal 75(17.5%).

Mother's education

A total of 422 children aged 6- 59 months with their mothers/caretakers were included in the study giving response rate of 100 %. Overall, 45.3% of mothers/caretakers of children had no formal education, 26.5% had primary education and secondary and above accounts 28.2%.

Accordingly, multivariate logistic regression analysis revealed that children whose mother had no formal education were 1.74(1.00-3.02) times more likely to be anemic than children of mother with secondary and above education level. Mother's education level was statistically significantly associated [p=0.051] with anemia.

The remaining maternal factors did not show statistically significant association with child anemia (Table 5).

Residence

Among study participants, 62.3% were living in urban area. Of these children, 38.4% were anemic and 48.6% non-anemic. Bivariate logistic regression shows that living in rural area was significantly associated with anemia [p=0.047]. Children who live in rural area were 1.5 times more likely to be anemic than their urban counterparts (Table 5).

Intestinal parasites

As displayed in Table 5, among the 77 children who were positive for intestinal parasites 41(53.2%) were anemic. Anemia was significantly associated with intestinal parasites [p-0.024].

Bivariate analysis showed that children who had intestinal parasites were 1.77(1.1, 2.9) times more likely to be anemic than those who had not. Reciprocally, children who had no intestinal parasites had 43.6% lower chance of getting anemia than children infected by intestinal parasites.

Ascaris lumbricoides

Of a total of 422 children 47 (11.1%) were positive for *Ascaris lumbricoides*. Among anemic children 29 (61.7%) were positive for this parasite. The study revealed the presence of Ascaris in the patient increases the chance of getting anemia [p-0.004]. Bivariate logistic regression shows that presence of Ascaris had statistically significant association with anemia, 2.49 (1.33-4.68) (Table 5).

Table 5. Factors Associated with anemia among children aged 6-59 months in Hawassa University Teaching and Referral hospital and Adare Hospital, South Ethiopia, 2016

Socio demographic Characteristics	Frequency	Anemic	Non-anemic	p-value	COR [95%CI]
Age					
6-23	174(41.2)	85(48.9)	89(51.1)	**0.040***	**1.8[1.02-3.15]**
24-35	98(23.2)	39(39.8)	59(60.2)	0.490	1.25[0.67-2.32]
36-47	75(17.8)	26(34.7)	49(65.3)	1.000	1.00[0.51-1.96]
48-59	75(17.8)	26(34.7)	49(65.3)		1
Sex					
Male	235(55.7)	98(41.7)	137(58.3)	0.998	1.0[0.7-1.5]
Female	187(44.3)	78(41.7)	109(58.3)		1
Religion					
Orthodox	137(32.5)	54 (39.4)	83(60.6)	0.430	1.6[0.5-5.4]
Muslim	117(27.7)	58(49.6)	5(50.4)	0.147	2.4[0.7-8.6]
Protestant	154(36.5)	60(39)	94(61)	0.447	1.6[0.5-5.3]
Catholic	14(3.3)	4(28.6)	10(71.4)		1
Residency					
Urban	284(67.3)	109(38.4)	175(61.6)		**1**
Rural	138(32.7)	67 (48.6)	71(51.4)	**0.047***	**1.5[1.0-2.3]**

Occupation					
Housewife	140(33.2)	67(47.9)	73(52.1)	0.633	0.7[0.1-3.2]
Employed	116(27.5	34(29.3)	82(70.7)	0.140	0.3[0.1-1.5]
Small scale business	79(18.7)	36(45.6)	43(54.4)	0.559	0.6[0.1-2.99]
Farmer	80(19)	35(43.8)	45(56.2)	0.498	0.6[0.1-2.8]
Others	7 (1.7)	4(57.1)	3(42.9)		1
Educational status					
No formal education	191(45.3)	91(47.6)	100(52.4)	0.023*	1.73[1.07-2.77]
Primary	112(26.5)	44(39.3)	68(60.7)	0.447	1.2[0.7-2.1]
Secondary and above	119(28.2)	41(34.5)	78(65.5)		1
Age group in year					
<30	241(57.1)	100(43.1)	132(56.9)	0.520	1.1[0.8-1.7]
>=30	181(42.9)	76(40)	114(60)		1
Weaning period					
≤6mth	172(40.8)	74(43)	98(57)	0.649	1.0[0.7-1.6]
>6mth	250(59.2)	102(40.8)	148(59.2)		
Number of children <5yr					
1	159(37.7)	58(36.5)	101(63.5)	0.091	0.7[0.5-1.1]
≥ 2	263(62.3)	118(44.9)	145(55.1)		
History of IP in the past 2 weeks					
Yes	106(25.1)	49(46.2)	57(53.8)	0.276	1.3[0.8-1.99]
No	316(74.9)	127(40.2)	189(59.8)		
History of malaria infection					
Yes	8(1.9)	5(62.5)	3(37.5)	0.242	2.3[0.6-10]
No	414(98.1)	171(41.3)	243(58.7)		1
Malaria Treatment in the last 3wks					
Yes	5(1.2)	2(40)	3(60)	0.938	0.9[0.2-5.6]
No	417(98.8)	174(41.7)	243(58.3)		1
Ascaris lumbricoides					
Yes	47(11.1)	29(61.7)	18(38.3)	**0.004***	**2.49[1.3-4.7]**
No	375(88.9)	147(39.2)	228(60.8)		1

Hook worm Yes No	 17(4) 405(96)	 7(41.2) 169(41.7)	 10(58.3) 236(58.3)	 0.964	 0.97[0.4-2.6] 1
E. histolytica Yes No	 18(4.3) 404(95.7)	 9(50) 167(41.3)	 9(50) 237(58.7)	 0.468	 0.7[0.3-1.8] 1
Intestinal parasites Yes No	 77(18.2) 345(81.8)	 41(53.2) 135(39.1)	 36(46.8) 210(60.9)	 **0.024**	 1.77[1.07-2.91] 1

Those variables with P -value of 0.25 or less in the bivariate logistic regression were candidate variables for the multivariate logistic regression model to control for possible confounding factors. Accordingly, as shown in Table 6, being in the age group 6-23 months compared to those aged 48-59 months and having parent/guardian with no formal education compared to those in secondary and above level of education were more likely to be anemic. The respective AOR and CI were 2.04[1.13-3.69, p=0.017] and 1.74 [1.00-3.02, p=0.051].

Table 6. Multivariate logistic regressions of selected variables associated with anemia among under five children in Hawassa University Teaching and Referral hospital and Adare hospital South Ethiopia, 2016.

Risk factor	Anemia	Unadjusted(COR)		Adjusted(AOR)	
	N (%)	OR[95%CI]	P -Value	OR[95%CI]	p-value
Child age					
6-23	85(48.9)	1.8[1.0-3.1]	0.040	**2.04[1.13-3.69]**	**0.017**
24-35	39(39.8)	1.2[0.7-2.3]	0.490	1.52[0.78-2.91]	0.211
36-47	26(34.7)	1[0.5-1.9]	1.000	1.11[0.55-2.24]	0.759
48-59	26(34.7)	1		1	
Residence					
Urban	109(38.4)	1		1	
Rural	67(48.6)	1.5[1.0-2.3]	0.047	1.24[0.76-2.02]	0.378
Mother.edu.level					
No formal	62(48.1)	1.67[1.0-2.7]	0.033	**1.74[1.00-3.02]**	**0.051**
Primary	57(42.9)	1.4[0.8-2.2]	0.207	1.43[0.82-2.52	0.208
Secondary or above	57(35.6)	1		1	
A. lumbricoides.					
Yes	29(61.7)	2.5[1.3-4.7]	0.004	2.44[0.93-6.46]	0.071
No	147(39.2)	1		1	

6.5. Associated risk factors of severe anemia

The prevalence of severe anemia among study participants was 16.1%. Socio-demographic data and clinical findings of severely anemic children were described in Table 7.

Among those children with severe anemia, most of them were within the age group of 24-35 which is 22.4%. The study also indicated that among under five years children who are living in rural area 30 (21.7%) were severely anemic and 43(17.2%) of children start complimentary food after six months and their hemoglobin was less than 7gm/dl.

Among a family who have more than two under five child, 42 (16%) of children showed severe anemia. Bivariate logistic regression shows factors associated with severity of anemia with statistical significance were residence (p=0.030) and age of child (p=0.026) (Table 7).

Table 7. Socio- demographic characteristics and clinical findings associated with severity of anemia among under five children at Hawassa University Teaching and Referral hospital and Adare hospital, Hawassa South Ethiopia, 2016.

Socio demographic Characteristics	Severe anemia N (%)	Others	Total	p-value
Age				
6-23	30(17.2)	144(82.8)	174	0.113
24-35	22(22.4)	76(77.6)	98	0.026
36-47	9(12)	66(88)	75	0.596
48-59	7(9.3)	68(90.7)	75	
Sex				
Male	40(17.0)	195(83)	235	0.570
Female	28(15)	159(85)	187	
Residency				
Urban	38(13.4)	246(86.6)	284	0.030
Rural	30(21.7)	108(78.3)	138	
Occupation				
Housewife	24(17.1)	116(82.9)	140	0.447
Employed	13(11.2)	103(88.8)	116	0.193
Small scale business	13(16.5)	66(83.5)	79	0.426
Farmer	16(20)	64(80)	80	0.594
Others	2(28.6)	5(71.4)	7	
Educational status				
No formal education	37(54.4)	154(43.5)	191	
Primary	17(25)	95(26.8)	112	0.082
Secondary and above	14(20.6)	105(29.7)	119	0.448
Age group in year				
<30	36(15.5)	196(84.5)	232	0.713
>=30	32(16.8)	158(83.2)	190	
Weaning period				
≤6mth	25(14.5)	147(85.5)	172	0.465
>6mth	43(17.2)	207(82.8)	250	

Number of children <5yr 1 ≥ 2	 26(16.4) 42(16)	 133(83.6) 221(84)	 159 263	 0.596
History of IP in the past 2weeks Yes No	 20(18.9) 48(15.2)	 86(81.1) 268(84.8)	 106 316	 0.374
History of malaria infection Yes No	 3(37.5) 65(15.7)	 5(62.5) 349(84.3)	 8 414	 0.115
Malaria Treatment in the last 3weeks Yes No	 0(0.0) 68(16.3)	 5(100) 349(83.7)	 5 417	 0.999
Ascaris lumbricoides Yes No	 11(23.4) 57(15.2)	 36(76.6) 318(84.8)	 47 375	 0.153
Hook worm Yes No	 2(11.8) 66(16.3)	 15(88.2) 339(83.7)	 17 405	 0.621
E. histolytica Yes No	 3(16.7) 65(16.1)	 15(83.3) 339(83.9)	 18 404	 0.948
Intestinal parasites Yes No	 16(20.8) 52(15.1)	 61(79.2) 293(84.9)	 345 77	 0.220

NB, others (mild, moderate)

7. DISSCUSION

In spite of the numerous interventions in place to control anemia in Ethiopia, the prevalence of anemia among under-five children is still high and classified as a severe public health problem according to WHO's classification (15). The general objective of this study was to determine the magnitude, severity and associated factors of anemia among under-five children in Hawassa. This was the first study conducted at Hawassa University Teaching and Referral hospital and Adare hospital to measure different factors thought to be associated with anemia.

The findings of this study indicated that anemia is a major public health problem among children under five years attending OPD and emergency units of Hawassa University Teaching and Referral hospital and Adare hospitals. On the basis of the WHO classification for persistent anemia in a population (40%), our findings confirmed that anemia among under five children was a severe public health problem.

The logistic regression estimates identified a significant relationship between the prevalence of anemia in children and a set of socio-demographic variables and odds ratio with 95% CI was computed to assess the strength of association.

Accordingly, the overall prevalence of anemia was found to be 41.7%, where mild, moderate and severe anemia being 6.6%, 19% and 16.1% respectively. The prevalence of anemia in this study is comparable with findings in Bangladesh 40.9% conducted in 2010 (28), 40.0% North Kazakhstan (29) and national prevalence EDHS 2011 (44%) (7) But the finding is lower than that of estimated global anemia prevalence (47.4%) (1) , and those reported from Ghana 78.4% in 2014 (36),Cape Verde 51.8% in 2014 (21), Northern part of Ethiopia 50.3% (38), Nigeria 70.5% in 2012 (32), Tanzania 77.2% in 2015 (20) Kenya 71.8% in 2005 (8) while it is higher than the study conducted in eastern Cuba in 2011which is 26% (50) , 28.8% from Kenya in 2014 (5) and 38.8% Haiti 2013 (31).The possible reason for these differences may be due to geographical difference, intervention used and the study period.

Age of the child being 6-23months was found to be significantly associated with anemia (P-value<0.05). A child whose age is less than 23 months was found to be 2.04 times more prone to anemia as compared to 48-59 months old counterpart [OR: 2.04(1.13, 3.69)].

This finding of declining of anemia as the child gets older is similar to studies conducted in southern Cameroon in 1998 (18) in northern Ethiopia 2014 (37), Tanzania in 2015(20). The high prevalence of anemia among infants and children less than 2 years of age also related to low concentration of iron in breast milk which may be insufficient to meet the daily iron requirements of the infant. In addition, the introduction of complementary foods often occurs within this period which is also a period for rapid physical development with increased blood volume and a decrease in iron storage from maternal source (33). Increasing age of children also decreases the chance of inter-current illness which may predispose to anemia (37).

Mother's education

Having parents/guardians with secondary education and above had a protective effect on the risk of anemia for their children.

Mothers' /guardians' level of education may positively influence practices related to the health care and feeding practice of their children. Educated mothers are more conscious of their children's health and introducing scientifically proved feeding practices, which help to improve their children nutritional status (16).

This result is comparable with studies in northeast Ethiopia 2015 (17) , Burma 2012 (11), Tanzania 2015 (20), Brazil 2013 (23), India 2014 (14) which have found that children whose mothers had no formal education were more anemic than those whose mothers had secondary and above level of education. The possible reason could be education has a relationship with the capacity to grasp the knowledge needed for adequate healthcare and nutrition for children, just as it provides a chance to enter the labor market and probably attain better socioeconomic conditions (16).

Others have also demonstrated that Mother's education level to have a protective effect on the chance of the child being diagnosed with anemia. Children whose mothers' had secondary, and higher levels of education, were less likely to be anemic (5).

On the other hand, the study conducted in Kilte Awulaelo Woreda, Northern Ethiopia shows maternal educational status were not associated with anemia among children aged 6–59 months old (17).

Residence

In our finding anemia was more prevalent among rural children (48.6%) than urban children (38.4%). The study conducted in Nigeria also support our finding, in that anemia was much more prevalent in rural (78.7%) than urban (61.3%) residents (33). The finding of the current study also concurs with the survey conducted among 6-59 months of children by EDHS in 2011 which shows that the prevalence of anemia is higher in rural area (45%) than urban area (35%), though the frequency of anemia was lower than our finding (38). The later can partly be explained by differences in the type of studied population that is health facility based in our case.

Based on the current finding children who live in urban area, had 34% less chance of having anemia than their corresponding children living rural area 0.660 [(95% CI=0.43-0.99)]. The study conducted in Bangladesh also indicated that anemia was higher in rural area (51.4%).The main reasons for the differences could be that lower consumption of iron in rural areas is due to poverty, ignorance, illiteracy, low socio-economic condition, inappropriate family planning, poor sanitation and unsafe drinking water in rural area (28).

But the study conducted in India contradicts with our study finding which reported no impact of place of residence on anemia (14).

***Ascaris lumbricoides* infection**

Fecal specimens were examined for the presence of helminthic larvae or eggs. *Ascaris lumbricoides* was the predominant intestinal parasite in this study with a prevalence rate of (11.1%). This finding is lower than the report from Gondar (2015) *(35.8%)*, and western Kenya (2005)19.3%. Parasitic infection has been identified as one of the main cause of anemia. Children playing habit and the transmission way of the parasite are the main factors facilitating the spread of infection. When children play they can easily acquire the infection as this parasite is soil transmitted helminthes (9). These variations in prevalence might be due to differences in climatic conditions, environmental sanitation, economic and educational status of parents.

In the current study children who are infected by *Ascaris lumbricoides* had a 2.5 times higher chance of getting anemia than non-anemic children.

Intestinal parasites

The prevalence of IPs found in this study was 77 (18.2%) which is lower than the prevalence reported in other studies from the same area Hawassa 26.6% (2015) (44). In our finding 26 (14.9 %) children aged below 23 months were positive for IPs, whereas the prevalence among those aged 24 months and above was 32 %. The higher prevalence of IPs in children aged ≥23 months as compared to children aged <23 months might be explained by the fact that older children have contact with faecally-contaminated soil while playing, which could predispose them to intestinal parasites.

The study conducted in Burma indicated that drinking un-boiled water can easily cause a parasite infection that could be the cause of intestinal bleeding induced anemia (11). In our finding anemia was significantly associated with intestinal parasites [p-0.024]

The likelihood of acquiring anemia in children who have any of the detected intestinal parasites was 1.77 [95% CI = 1.1-2.9] times higher than those who had no intestinal parasites. The reason for this may be due to intestinal parasites suck blood and also damage the intestinal wall, causing blood leakage. Intestinal parasite have been associated with anemia in several studies including studies by Nyaruhucha *et al*.,2005 (22), and Semedo *et al* 2014 (21). Another finding also confirms that intestinal worms are one of the reasons for anemia among children in India (14).

Severity of anemia

In this study the magnitude of severe anemia among the study participants was 16.1%. This result is somewhat lower than the study conducted in Tanzania 27.7% (20). Malaria in children is attributed as a cause of severe anemia; however, in the current study there is no child infected by malaria parasites as assessed by examining both thin and thick smears.

But on the other hand our result was higher than findings in western China among children under 36 months which documented 3.2% severe anemia (30); this can be due to variation of age category, which is restricted to under 36 months in the Chinese study. In this study, severity of anemia significantly associated with residence P- [0.030]. Those children living in rural area were highly susceptible to sever anemia. The odds of anemia in rural children was 1.79 times higher [1.79 (95%CI, 1.06-3.05)] than urban area. Multivariate logistic regression does not show any statistical significant association. This study also found a significant

association between age above two years and severe anemia in bivariate analysis [p-0.026] but not in multivariate analysis. Similar finding was reported in Tanzania (18).

Direct association between intestinal parasites and age is likely to be the cause of this in our study. Prevalence of intestinal parasites increased with age, 64.2% in children older than 23 months versus 14.9% in children less or equal to 23 months.

8. STRENGTH AND LIMMITATION OF THE STUDY

8.1. Strength of the study

- ✓ studying magnitude of anemia and associated risk factors (both laboratory based and questionnaire based) in the neglected age group
- ✓ Analyzing stool using both direct and concentration methods

8.2. Limitation of the study

- ✓ Anemia was measured based on hemoglobin concentration only, inclusion of other measures such as serum ferritin could lead to better diagnosis.
- ✓ Being a hospital based study, the result cannot be extrapolated to the larger community
- ✓ The cross-sectional nature of the study. Follow-up studies provide more insight into public health problems.

9. CONCLUSION AND RECOMMENDATIONS

9.1. Conclusion

- Burden of anemia among children aged 6–59 months in the study site is high (41.7%) and it has severe public health significance according to the WHO cut-off points.
- Anemia was of mild, moderate and severe type in 6.6%, 19% and 16.1%, respectively.
- Age of child, mother with no formal education, living in rural area, having intestinal parasites and specifically *Ascaris lumbricoides* significantly associated with anemia.

9.2. Recommendations

Based on the findings the following recommendations are made:

- Based on the identified risk factors, the approach to anemia control should ensure that preventive strategies be targeted to all under five children irrespective of their hemoglobin level or clinical status.
- Environmental sanitation activities needs to be strengthened
- Continuous health education program should be developed after assessing caretakers' knowledge, practices and beliefs on anemia in children.

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11. ANNEXES

Annex I: Questionnaire

Addis Ababa University, College of Health Sciences, School of Allied Health Science, Department of Medical Laboratory Sciences

Questionnaire: to assess the prevalence of anemia and associated risk factors among under five children attending Hawassa University Teaching and Referral hospital and Adare hospitals, Hawassa Southern Ethiopia.

Socio-demographic variables

1. Participant code _____
2. Age of child (months) ----------
3. . Sex
   1. Male 2. Female
4. Religion
   1. Orthodox 2. Muslim 3. Protestant 4.catholic 5. Others (Specify) _____
5. Mother's education
   1. No formal education
   2. Primary
   3. Secondary and above
6. Mother's occupation
   1. Housewife
   2. Employed
   3. Small scale business
   4. Farmer
7. Introduction of complementary foods Age of:
   1. ≤ 6 month 2. > 6 months
8. Residence
   1. Urban 2. Rural
9. Anthelmintic intake in the past 6 months
   1. Yes 2.No

10. Number of children <5 years
    1. One
    2. Two or more
11. Age of mother years.
12. History of malaria infection
    1. Yes 2. No
13. History of intestinal parasite in the past 2weeks
    1. Yes 2. No
14. Antimalarial drug in the last three months
    1. Yes 2.no

Name of the interviewer _____ _____

Signature _____ Date _____

Questionnaire /Amharic version/

አዲስአበባ ዩኒቨርሲቲ የህብረተሰብ ጤናና ሕክምና ሳይንስ ኮሌጅ የሜዲካል ላቦራቶሪ ሳይንስ ትምሕርት ክፍል

ይህ ቅጽ የደም ማነስ ስርጭትና ተያያዥ ችግሮ ከዓምስት አመት በታች በሆኑ ህጻናት ላይ ተጋላጭ የሚያደርጉ ምክንያቶችን መሰብሰቢያ ነዉ።።

1. የተሳታፊው ልዩ መለያ_____

2. ዕድሜ (በወር)

3. ፃታ

    ወንድ-------- ሴት----------

4. ሐይማኖት
    1. ክርስቲያን
    2. ሙስሊም
    3. ፕሮቴስታንት
    4. ሌላ (ይግለጹ)

5. የእናት የትምህርት ደረጃ

1. ያልተማረ

2. የመጀመሪያ ደረጃ

3. ሁለተኛ ደረጃ እና ከዚያ በላይ

6. የእናት የስራ ዓይነት

የቤት እመቤት

ተቀጣሪ

ጥቃቅንና አነስተኛ

ግብርና

7. ተጨማሪ ምግብ የተጀመረበት እድሜ

1. ከ 6 ወርበፊት

2. ከ 6 ወር በሁዋላ

8. መኖሪያ

1. ከተማ 2. ገጠር

9. ባለፉት ስድስት ወራት ለሆድ ዉስጥ ትላትል የሚሆን መድሀኒት ወስደዋል?

1. አዎ 2. አይደለም

10. ከ 5 ዓመትበታች የሆኑ ልጆቸ ብዛት

1. አንድ 2. ሁለትእናከዚያበላይ

11. የእናት እድሜ _____

12.በወባ በሽታ ታመው ያውቃሉ

1. አዎ 2. አይደለም

13. ባለፉት 2 ሳምንታት በሆድ ውስጥ ጥገኛ ትላትል ታሞ ነበር

1. አዎ 2. አይደለም

ነ4. ባለፉት ሶስት ወራት የወባ መድሃኒት ወስደዋል ?

1. አዎ 2. አይደለም

የጠያቂው ስም ፊርማ ቀን ----------

የዋና ተመራማሪ ----------

Annex II: Information sheet for study participants

Investigator: Yeshimebet G/selassie

Advisors:

Aster Tsegaye, PhD

JemalAlemu BSc, MSc

Name of organization: Addis Ababa University

Title of the study

Prevalence of anemia and associated risk factors among under five children attending at Hawassa University Teaching and Referral hospital and Adare hospitals, Southern Ethiopia, December 2015 to May2016.

Purpose of the study

The objective of this study is to determine the prevalence of anemia and associated risk factors among under five children attending at Hawassa University Teaching and Referral hospital and Adare hospitals.

Procedures to be carried on the study subjects

1. About 2 ml venous blood will be collected
2. Each individual will be provided with labeled clean plastic container, toilet tissue paper and pieces of applicator sticks to collect stool sample

Risks associated with the study

There is no risk on the study participants but the child may have discomfort related to blood collection

Benefits of the study

The study finding will be used to identify the status of anemia this can be used to treat anemic children appropriately. It will also provide information on the associated factor of anemia which can help to control the main cause of anemia in under five children. Findings

from this study will help us in setting prevention programs and developing treatment protocols.

Confidentiality of your information

All information obtained will be kept private

Voluntary participation

Participation is only by willingness. You have the right to withdraw your child from the research at any time without in any way affecting his/her further medical care. I would also like to inform you that this study will be approved by Addis Ababa University research ethical board.

If you have any question you can contact:

The address of investigator: Yeshimebet G/Selassie
Email yeshigd@ yahoo.com
Mobile 0911063022
The address of advisors of this study is:

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Jemal Alemu BSc, MSc

Tel: 0911429989

Address of the Department of Medical Science Research Ethics Committee office

Tel +251112755170

Information sheet for study participants /Amharic Version/

ክፍል አንድ፡- ለጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ

ዋና ተመራማሪ የሺመቤት ገ/ስላሴ

አማካሪዎች፡ዶ/ር አስቴር ጸጋዬ

የድርጅቱ ስም: አዲስአበባ ዩኒቨርሲቲ

የጥናቱርዕስ፡ የደም ማነስ ስርጭትና ተያያዥ ችግሮች ከዓምስት አመት በታች በሆኑ ህጻናት ላይ

ጤና ይስጥልኝ፡፡እኔ_____እባላለሁ፡፡

ይህንን ጥናት የምሰራው አዲስአበባ ዩኒቨርሲቲ የድህረ-ምረቃ ትምህርቴን ለማጠናቀቅ ማJያ እንዲሆነኝ ነዉ።

የጥናቱዓላማ

የደም ማነስ ስርጭትና ተያያዥ ችግሮች ከዓምስት አመት በታች በሆኑ ህጻናት ላይ ለማዎቅ የሚረዳ ጥናት ነው፡፡

የአሰራሩ ሂደት

በዚህጥናት ዉስጥ ይሳተፉ ዘንድ እንጠይቆታለን፡፡ ለመሳተፍ የደምና የሰገራ ናሙና መስጠትና ከበሽታው ጋር ተያያዥ የሆኑ መጠይቆችን መመለስ ይጠበቅብዎታል።

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

በጥናቱ መሳተፍ ምንም አይነት ተያያዥ ችግር አያስከትልም።

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

በሽታው ከተገኘ የህክምና እርዳታ እንዲያገኙ ይደረጋል፡፡

ክፍያን በተመለከተ

በጥናቱ በመሳተፍ ምንም አይነት ክፍያ አይከፈልዎትም። በአንጻሩ ከልብ ልናመሰግንዎት እንወዳለን፡፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ዉስጥ የተሰበሰቡ ማናቸውም መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡

ከጥናቱ ስለመውጣትና ስለማቋረጥ

ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛውም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ፡፡ ከጥናቱ ቢወጡም እንኳን የተለመደውን የህክምና እርዳታ በጤና ተቋሙ ውስጥ በማንኛውም ጊዜ የማግኘት መብት አለዎት፡፡

ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎት በሚከተለው አድራሻ ጥያቄዎን ማቅረብ ይችላሉ።

ወ/ሮ የሺመቤት ገ/ስላሴ (ስልክ:0911063022) ፣አዲስአበባ ዩኒቨርሲቲ፣ ኣዲሰአበባ፣ ኢትዮጵያ፡፡

Annex III: Consent form for study participants

Name: _____

I have read the information sheet (or it has been read to me); I have understood that it involves about prevalence of anemia and associated risk factors among under five children attending at Hawassa University Teaching and Referral hospital and Adare hospitals. And also I have understood about the purpose of the study, procedures to be carried out, risks associated with the study, benefits of the study and confidentiality of the information. Therefore I consent voluntarily to my child's participation as a participant in this research and understand that I have the right to withdraw my child from the research at any time without in any way affecting his/her further medical care I have given my consent freely to participate in the study.

I_____ hereby give my consent for giving of the requested information, blood and a stool samples for the study.

Parent /guardian signature_____ Date_____

Investigator's signature _____Date _____

Consent form for study participants/Amharic version/

ክፍል ሁለት- የስምምነት ቅጽ

የተሳታፊው መለያ ቁጥር_____

የተሳታፊው ህጻን ወላጅ/አሳደጊ ሙሉ ስም_____

እኔየ_____ወላጅ/አሳዳጊ ልጄ በዚህ ጥናት እንዲሳተፍ/እንድትሳተፍ/ እና የደምና የሰገራ ናሙና እንዲሰጥ ተጠይቄዐለሁ፡፡ በክፍል አንድ ዉስጥ ያለዉ መረጃ ተነቦልኛል፡፡ መጠየቅ የምፈልገዉን ጥያቄ በአግብቡ ጠይቄ አስፈላጊዉን ምላሽ በአጥጋቢ ሁኔታ ተመልሶልኛል፡፡ ስለሆነም በፈቃደኛነት ልጄ በጥናቱ ተሳታፊ እንዲሆን/እንድትሆን/ እና የደምና የሰገራ ናሙና ሰጥቶ/ታ/ የደምማነስ ምርመራ እንዲደረግበት መፍቀዴን በፊረማዬ አረጋግጣለሁ።

የወላጅ/ያሳዳጊ ፊርማ _____

የዋና ተመራማሪ ፊርማ_____

Annex IV: Laboratory report form

Serial no_____

Date of specimen collection:_____time:_____

Stool examination

Microscopic examination

Direct (wet mount): _____

Concentration technique: _____

Blood examination

a. Malaria parasites

b. CBC examination report

Annex V: Laboratory techniques

Collection and preparation of thick malaria smear (48)

1. Hold the third finger of the left hand of the patient between your left thumb and finger at the first phalangeal joint.
2. Wipe fingertip with swab dipped in antiseptic solution.
3. Allow the fingertip to dry.
4. Hold the pricking needle in right hand and prick the finger and allow blood drop to ooze out.
5. Take a clean, dust free, grease free slide and take 3 drops of the blood 1 cm from the edge of the glass slide.
6. Make thick smear by joining the 3 drops of blood and spreading it in an area of 10 mm diameter.
7. Allow it to air dry.

Giemsa staining thick blood smear for hemo-parasites(48)

This is recommended for detection and identification of blood parasites.

Procedure:

1. Dilute Giemsa stock solution to 1:10 using buffer ph 6.8.
2. Place blood smear on a staining rack facing upward.
3. Flood smear with freshly dilute giemsa stain for 15 minutes.
4. Wash with clean water using a wash bottle.
5. Put stained smear on a standing rack and leave to air dry.
6. Stained blood smear is now ready for microscopic examination.

Wet mount /direct examination of faecal specimens(49)

1. Place one drop of 0.85% NaCl on the slide.
2. Take a small amount of faecal specimen and thoroughly emulsify the stool in saline
3. Place a 22mm cover slip at an angle into the edge of the emulsified faecal drop.
4. Systematically scan the entire 22mm cover slip with the 10x objective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs.

Formol-ether concentration technique (49)

1. Using a stick, emulsify an estimated 1g of faeces in about 4ml of 10% formol water contained in a screw –cap bottle or tube.
2. Add further 3-4ml 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.
5. Stopper the tube and mix for 1 minute.
6. With a piece of wrapped around the top of the tube, loosen the stopper.
7. Centrifuge immediately at 3000 rpm for 1 minute.
8. Using a stick, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, fecal debris and formol water.
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.
10. Transfer the sediment to the slide, and cover with cover glass.
11. Examine the preparation microscopically using the 10x objective with the condenser closed sufficiently to give good contrast. Use 40X objectives to examine cysts.

Annex VI: Procedure and reagents of Cell-Dyn 1800 Hematology Analyzer

Figure: 4 Cell Dyn 1800 hematology analyzer

Annex VII: Procedure for cell Dyn 1800 hematology analyzer

1. Whole blood collected in an EDTA tube with a Minimum sample volume is 0.5 ml using the Open Sample Mode. The instrument aspirates 30 µl of patient sample.
2. . Run three levels of QC at the beginning of each day of patient testing. Do not perform patient testing until QC tests are performed and within acceptable limits. Rerun at least one of the three levels of QC again after eight hours of patient testing to assure the instrument is still functioning properly.
3. Press MAIN to return to the MAIN MENU. At the MAIN MENU, enter in the operator ID and press RUN, next press SPECIMEN TYPE. If the instrument has been idle for fifteen minutes or more, press normal background. Press the Touch Plate to run an Open Mode Background test. Verify that the Open Mode Background count results are acceptable.
4. Press MAIN to return to the MAIN MENU screen. Enter in the Operator ID and press RUN. Press SPECIMEN TYPE then press PATIENT SPECIMEN. Verify that RUN Ready is displayed in the Status Box. Scan patient specimen number and patient name using the keyboard. Expected ranges for blood counts differ based on gender and age.

5. The Cell-Dyn is programmed to display the correct reference range. The operator, however, must first manually type in the correct gender prior to running the patient sample. Once RUN Ready is displayed in the Status Box, use the ↓key to scroll to the Limit prompt. Enter either "1" for Male or "2" for Female. Mix the patient sample well and remove the cap. Place the sample probe in the tube so that the end is immersed in the sample but not resting on the bottom of the tube.

6. Press the Touch Plate to start the run. The Status Box on the RUN menu indicates the stage of the run. When Remove Specimen is displayed in the Status Box and the probe has moved up through the wash block remove the sample tube and replace the tube cap.

7. A beep will indicate that the probe cleaning cycle has begun. After the probe cleaning cycle is complete, the probe will move down into position for the next sample and the results will be displayed on the screen.

8. If needed, press PRINT REPORT for a hardcopy of the report. After sampling is complete, press MAIN to return to the MAIN MENU. Change the Operator ID to

Annex: VIII Reagents for Cell-Dyn 1800

1. Cell-Dyn Diluents:
    - ✓ Stable at room temperature until the expiration date on the container.
    - ✓ Protect from direct sunlight, extreme heat, and freezing during storage
    - ✓ Do not use if reagent has been frozen.

2. Cell-Dyn Lytic Agent:
    - ✓ Stable at room temperature until the expiration date on the container.
    - ✓ Protect from direct sunlight, extreme heat, and freezing during storage
    - ✓ Do not use if reagent has been frozen.

3. Cell-Dyn Detergent:
    - ✓ Stable at room temperature until the expiration date on the container
    - ✓ Protect from direct sunlight, extreme heat, and freezing during storage.
    - ✓ Do not use if reagent has been frozen.

4. Enzymatic Cleaner:
    - ✓ Stable at 2-8°C until the expiration date on the container.
    - ✓ Do not use if reagent has been frozen.

5. Cell-Dyn Whole Blood QC:
    - ✓ Unopened QC vials are stable at 2-8°C until the expiration date on the vial.

    Opened QCvials are Stable at 2-8°C for 7 days after opening. Do not use expired QC.
    - ✓ Allow QC to sit at room temperature for fifteen minutes before testing.
    - ✓ Mix QC vial by rolling the vial between palms for 20 seconds.
    - ✓ Invert the vial and roll it back and forth for another 20 seconds.
    - ✓ Gently invert the vial 10 times.
    - ✓ Do not shake.
    - ✓ Continue to mix in this manner until cells are completely suspended (3-5 times).
    - ✓ Gently invert the pre-mixed vial 5 times immediately before testing.
    - ✓ Return vial to refrigerator when testing is complete.

6. Whole Blood Calibrator:
    - ✓ Unopened calibrator vials are stable at 2-8°C until the expiration date on the vial.

- ✓ Opened calibrator vials are stable at 2-8°C for 7 days after opening. Do not use expired calibrators.
  - ✓ Allow the calibrator to sit at room temperature for fifteen minutes before testing.
  - ✓ Mix the calibrator vial by rolling the vial between the palms for 20 seconds.
  - ✓ Invert the vial and roll it back and forth for another 20 seconds.
  - ✓ Gently invert the vial 10 times.
  - ✓ Do not shake.
  - ✓ Continue to mix in this manner until cells are completely suspended (3-5 times).
  - ✓ Gently invert the pre-mixed vial 5 times immediately before testing.
  - ✓ Return vial to refrigerator when calibration is complete.

Declaration

I, the undersigned, declare that this M.Sc. 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 duly acknowledged.

M.Sc. candidate: **Yeshimebet G/Selassie (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Aster Tsegaye (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Jemal Alemu (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.