ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Hematological Profile of Pregnant Women Attending Antenatal Care at Dilla University Referral Hospital, Dilla, Ethiopia: Comparative Cross Sectional Study

By: Sara Anberbir (BSc)

Advisors: - Mintwab Hussein (MSc, BSc)

Melatwork Tibebu (MSc, BSc)

A thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology specialty track).

October, 2016

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

School of Allied Health Science

Department of Medical Laboratory Sciences

(Hematology and Immunohematology Specialty Track)

Hematological Profile of Pregnant Women Attending Antenatal Care at Dilla University Referral Hospital, Dilla, Ethiopia: a Comparative Cross Sectional Study

By: Sara Anberbir

Approved by the Examining Board	**Signature**
_____	_____
Chairman, Dep. Graduate Committee	**Signature**
_____	_____
External Examiner	**Signature**
_____	_____
Internal Examiner	**Signature**
_____	_____
Advisor	**Signature**
_____	_____
Advisor	**Signature**
_____	_____

Acknowledgment

My gratitude goes to God Almighty, the essence of my existence, all I have ever been and will ever be in life, I owe it all to you. I would like to acknowledge Addis Ababa University, College of Health Sciences, School of Allied Health Science and Department of Medical Laboratory Sciences for giving this opportunity. I wish to express my deepest gratitude to my advisors Ms. Mintwab Hussein and Melatwork Tibebu for their constructive comment and guidance from the development of the study proposal through the final write-up of the thesis.

My best regard extends to all staffs and study subjects of DURH without their cooperation it would have been impossible to accomplish the study. I would like to extend my deepest thanks to my father (Anberbir), mother (Genet), sister (Rita) and brothers (Abel and Yohannes) for their love, care, and help that enabled me to realize my long-term dream. Last but not least I would like to thank all my friends who have partly made their contribution in the development of the entire research project.

Table of contents

List of tables

List of figures

Abbreviation

ANC	Antenatal Care
DLC	Differential Leukocyte Count
DURH	Dilla University Referral Hospital
EDHS	Ethiopia Demographic and Health Survey
EDTA	Ethylene Diamine Tetra Acetic Acid
ESR	Erythrocyte Sedimentation Rate
fl	Femto liter
g/dl	gram per deciliter
Hb	Hemoglobin
HCT	Hematocrit
HIV	Human Immunodeficiency Virus
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
ml	Milli liter
PCV	Packed Cell Volume
Pg	Pecogram
PLT	Platelet
RBC	Red Blood Cell
RDW	Red Cell Distribution Width

SD	Standard Deviation
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
STH	Soil Transmitted Helminthes
TLC	Total Leukocyte Count
WBC	White Blood Cell
WHO	World Health Organization

Operational definitions

Anemia: Hb concentration less than 11 g/dl for pregnant women.

Hematological profile: Red blood cells, Red blood cell indices, Hematocrit, Hemoglobin, White blood cells, differential white blood cell and platelets value of pregnant women and it also includes morphology of cells.

Hypochromic: Red cell with a more prominent central pallor (i.e. greater than 1/3 the diameter of the cell).

Macrocytic: erythrocytes having large size (RBCs larger than the small lymphocyte nucleus) and MCV value greater than 100 fl.

Microcytic: abnormally small erythrocytes (Red cells smaller than the small lymphocyte nucleus) and MCV value less than 80 fl.

Mild anemia: when the hemoglobin concentration of pregnant women is between 10.0-10.9g/dl. Moderate anemia: when the hemoglobin concentration of pregnant women is between 7.0-9.9g/dl.

Normochromic: normally staining red blood cells (RBC with about 1/3 central pallor)

Normocytic: when red blood cells have normal size (RBC similar to size of small lymphocyte nucleus) and MCV value between 80 – 100 fl.

Severe anemia: when the hemoglobin concentration of pregnant women is less than 7g/dl.

Abstract

Background: Pregnancy manifests number of change in the normal physiology. Important of these changes are those which alter the hematological parameters. Hematological profile is considered one of the factors affecting pregnancy and its outcome. Anemia is the most common hematological problem in pregnancy.

Objective: To assess the hematological profile of pregnant women in comparison with non pregnant women (as a control).

Methodology: Comparative cross sectional study was conducted from March to May, 2016, on 110 pregnant and 110 non pregnant women at Dilla University Referral Hospital. A structured, pretested and interviewer administered questionnaire and laboratory tests were used to obtain data. Data was analyzed using the statistical soft ware SPSS version 20. Logistic regression, independent t test and one way ANOVA was used. P value less than 0.05 were taken as statistical significant.

Result: The result showed that pregnant women exhibited statistically significant decrease value of RBC, Hb, HCT, MCHC, Lymphocyte, MID and PLT counts when compared with non pregnant. White blood cell, neutrophils and MCH were significantly higher in pregnant women compared to non pregnant women. No significant difference in MCV and RDW of pregnant women compared to non pregnant women. There was a significant decrease in RBC, Hb and HCT in the 2^{nd} trimester compared to 1^{st} trimester pregnancy. The RDW value increased significantly in third trimester when compared with 1^{st} trimester pregnancy. The prevalence of anemia obtained in this study was 15.5%. Pregnant women who had primary educational level were 6.28 times more likely to be anemic compared to higher educational level.

***Conclusion*:** There was a significant difference in the majority of hematological parameters between pregnant and non pregnant women. RBC, Hb, HCT, MCHC, Lymphocyte, MID and platelet counts were significantly decreased whereas WBC, neutrophils and MCH were significantly increased in pregnant women. Therefore, there is a need to monitor these parameters during pregnancy and thus improve the outcome of pregnancy.

Key terms: Hematological profile, pregnant women, anemia

1. Introduction

1.1 Background

Hematological profiles are those related to blood and the blood forming organs. The values of these profiles, some of which includes Packed Cells Volume (Hematocrit), Red and White Blood Cells counts, Platelet count, Erythrocyte Sedimentation Rate, Differential Leukocytes count, etc., enables the health worker to reach one of several diagnosis and decisions [1].

It is known that the condition of a person can be interpreted based on hematological profile via comparing the individuals' blood data with the reference data. However, health of an individual indicated by blood parameters is known to be at different values in different countries, in the same country at different times and gender, and in same individuals at different situations, conditions and time. Hence, the blood value is not an absolute value but must be comparable with the reference range for an individual to be considered physiologically healthy [2].

Pregnancy is the period from conception to birth. After the egg is fertilized by a sperm and then implanted in the lining of the uterus, it develops into the placenta and embryo, and later into a fetus. Pregnancy usually lasts 40 weeks, beginning from the first day of the woman's last menstrual period, and is divided into three trimesters, each lasting three months [3].

Pregnancy is typically divided into three trimesters. The first trimester is from week 1 through 12 and includes conception. Conception is followed by the fertilized egg traveling down the fallopian tube and attaching to the inside of the uterus, where it begins to form the fetus and placenta. The second trimester is from week 13 through 28. The third trimester is from 29 weeks through 40 weeks. Pregnancy causes physiologic changes in all maternal organ systems; most return to normal after delivery. In general, the changes are more dramatic in multi-fetal than in single pregnancies [4].

Pregnancy manifests number of change in the normal physiology in order to meet the demand of the developing fetus and placenta. Important of these changes are those which alter the hematological parameters [5]. Plasma expansion and haemodilution during pregnancy contribute to majority of these changes [6, 7, 8]. Maternal blood volume increases during pregnancy and this involves an increase in plasma volume as well as in red cell and white cell volume. The

blood volume increase by 40% to 45% on average, this increase occurs faster in the second trimester [9].

The changes in the hematological status of pregnant women are profound. Modifications in the production of red cells and changes in plasma volume shift fundamental hematological indices such as red blood cell (RBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and white blood cell (WBC) count. Some of these are decreased – for example, RBC and PLT counts – partly as a result of the physiological haemodilution that occurs in pregnancy, while others are increased, such as the WBC count [10].

During pregnancy, the total blood volume increases by about 1.5 liter, mainly to supply the needs of the new vascular bed. Expansion of plasma volume by 25%–80% is one of the most marked changes, reaching its maximum by mid pregnancy. However, the increase in RBC mass has been found to be approximately 30% between the twelfth and thirty-sixth week of gestation when iron and folate are supplemented. The discrepancy between the rate of increase in plasma volume and that in RBC mass leads to physiological anemia. In late pregnancy, plasma volume increases at a slower rate, inducing a slight rise in hematocrit level. These physiological changes during pregnancy make it difficult to define normal hematological reference intervals for pregnant women [10].

Hemoglobin concentration falls. Typically, this is by 1–2 g/dl by the late second trimester and stabilizes thereafter. Women who take iron supplements have less pronounced Hb changes, as they increase their red cell mass proportionately more than those without dietary supplements (the increase is approximately 30% over pre pregnancy values). Hematocrit value is likewise lower in pregnancy [11, 12]

More than half of the pregnant women in the world have hemoglobin levels indicative of anemia. Knowledge of the current situation of the condition in our environment is necessary. This knowledge will motivate antenatal caregivers toward early detection and prompt management of anemia in pregnancy [13]. The demand of the developing fetus placed the expectant mother at greater risk for nutritional anemias, especially deficiencies of iron and folate [14].

A. Dilutional Anemia

The plasma volume increases disproportionately to red cell mass. A reduction in the hematocrit and hemoglobin concentration usually stabilizes at 0.33L/L and 11g/dl respectively. Serve a useful purpose by enhancing placental perfusion, thereby facilitating oxygen and nutrient delivery to the fetus. An additional benefit is that fewer red cells are lost with the hemorrhage accompanying placental separation [14].

B. Nutritional anemia

When the hemoglobin concentration is less than 10.4g/dl a true reduction in red cell mass is likely present; however, because of variation in the magnitude of the hydremia, a fixed dividing line between the normal and abnormal is difficult to place in pregnancy. Red cells remain normochromic and normocytic unless deficiency of iron or folate supervenes [14].

In developing countries multiple factors lead to nutritional anemia in pregnancy such as life style, low socioeconomic condition, illiteracy and lack of knowledge of good dietary habits. Anemia during pregnancy is associated with increased maternal morbidity and mortality, and contributes to 20% of the maternal mortality in Africa. Folate deficiency accounts for 95% of megaloblastic anemia in pregnancy. Iron deficiency anemia (IDA) is a major health problem during pregnancy [15].

The mean minimum acceptable hemoglobin level during pregnancy by World Health Organization (WHO) criteria is taken to be 11 g/dl. WHO further divides anemia in pregnancy into: mild anemia (Hb 10-10.9 g/dl), moderate anemia (Hb7.0-9.9 g/dl) and severe anemia (Hb<7 g/dl) [16].

The other red cell indices change little, although red cells show more variation in size and shape than in the non-pregnant state. There is a small increase in mean cell volume (MCV), of on average 4 fl for iron-replete women, which reaches a maximum at 30–35 weeks gestation and occurs independently of any deficiency of B12 and folate [11].

During pregnancy there is a general downward drift in PLT count, particularly during the last trimester. This results at term in a level that is approximately 10% less than the pre pregnancy level. The mechanisms for this are thought to be a combination of dilution effects and acceleration of PLT destruction across the placenta. Most women still have PLT counts within the normal range; however, if the starting count is at the lower end of the normal range, or there is a more severe drop, thrombocytopenia occurs (PLT count $<140\times10^9$/l) [17]. In over 75% of cases this is mild and of unknown cause, a condition referred to as incidental thrombocytopenia of pregnancy. Approximately 21% of cases are secondary to a hypertensive disorder will 4% are associated with immune thrombocytopenic purpura (ITP). No treatment is required and the infant is not affected. A pregnant woman will also become hypercoagulable, leading to increase risk for developing blood clots and embolisms, due to increase liver production of coagulation factors, mainly fibrinogen and factor VIII [18].

WBC is increased in pregnancy with a typical reference range of 6×10^9– 16×10^9/L. In the hours after delivery, healthy women have been documented as having WBC 9×10^9–25×10^9/L. This leukocytosis is mainly due to neutrophilia and immature forms like metamyelocytes and myelocytes (neutrophil left shift) may be present in the peripheral blood film. Monocytosis is also reported during pregnancy. By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (4×10^9–10×10^9) [11, 19].

The study of hematological parameters during pregnancy helps to differentiate between the physiological anemia of pregnancy, which is a result of haemodilution, which occurs normally during pregnancy and the pathological anemia [20].

1.2 Statement of the problem

Pregnancy is one of the most and unique periods of women's life cycle. Though it is the most exciting period of expectations and fulfillments, but it is a condition of great stress because many anabolic activities takes place and fetal growth is accomplished extensive changes in maternal body composition and metabolism [21].

Many physiological hematological changes occur during pregnancy to fulfill the demands of the developing fetus. While physiological in nature, but abnormal hematological profile does affect pregnancy and its outcome [22]. One of the most important underlying causes of maternal mortality is due to underlying hematological complications. Anemia and thrombocytopenia are the most frequent complication during pregnancy [5].

Anemia is a very common hematological problem in the developing and under developed countries particularly among vulnerable groups such as infancy, childhood and in women of reproductive age group [23]. It is a burning public health problem and most commonly encountered medical disorder during pregnancy. It increases the incidence of maternal mortality and is associated with increased fetal and infant mortality; prematurity and low birth weight [24]. Intrauterine growth retardation and low birth weight inevitably lead to poor growth in infancy, childhood and adolescence and contribute to low adult height [25].

Etiological classification of anemia identifies three major causative groups of anemia as nutritional, marrow disease and hemolytic disease. Anemia resulting from iron deficiency is the most prevalent form of anemia (76%), followed by foliate deficiency 20 % and combined iron and foliate deficiency 20 %. *Hookworm* infestation, malaria and HIV infections contribute greatly to severe anemia cases reported among pregnant women. It is evident that several factors may be implicated for the high prevalence of anemia in the pregnant population [12].

The impact of anemia on the future health of newborns is a cause of concern [26, 27]. Globally, anemia contributes to more than 115, 000 maternal deaths and 591,000 prenatal deaths every year [28].

The consequences of anemia can be quite severe and are often irreversible, affecting both human and socioeconomic health of individuals and families. Mild to moderate anemia leads to

weakened immunity, reduced work capacity, reduced cognitive ability and an overall decreased quality of life. Severe anemia (Hb <70g/L) reduces a woman's ability to survive bleeding during and after childbirth and is considered a major cause of maternal morbidity and mortality, particularly in the non industrialized world [29].

In Africa, 57.1%of the pregnant women were anemic. Although frequency differs widely in different settings and accurate data is often missing, however in areas with limited facilities significant proportions of childbearing women are anemic [16]. Geographically, those living in Asia and Africa are at the greatest risk. Moreover, there is also a large variation in the incidence of anemia during pregnancy because of the changes in socioeconomic conditions, lifestyles, and health seeking behaviors of various individuals across different countries and cultures and obstetrics and gynecological related situation of pregnant mothers [30].

According to the review conducted in 2014, anemia is one of the serious health problems among pregnant women in Ethiopia. Prevalence rates of 40.5% in the general population and 47.2% in the children were reported from southwest Ethiopia. Higher rates about 57% have also been reported in pregnant women in Jimma [31].

Malaria infection especially in the first and second trimesters has been implicated in adverse pregnancy outcomes. It causes 3% -5% of maternal anemia cases. About 50 million pregnant women are exposed to malaria especially in the high endemic regions [32].

Next to anemia, Thrombocytopenia is the second most common hematologic abnormality during pregnancy. It is encountered in 7-8% of all pregnancies [33].

In normal pregnancy the hematological indices of an individual to a large extent reflect their general health and many studies such as Akinbami *et al*. [10] and Osonuga *et al*. [34] have identified the hematological indices of the pregnant woman as one of the factors affecting pregnancy. Anemia is a widely identified hematological abnormality and it is also associated with adverse pregnancy outcome [35].

Successful outcome of pregnancy requires frequent monitoring of hematological parameters to avoid complications throughout the trimester of pregnancy. The prevalence of anemia in pregnant women's in Ethiopia has been studied in different times without addressing all the

hematological parameters such RBC indices, WBC, platelets and with no control group. In Dilla specifically, there are no documented studies on prevalence of Anemia and its associated factors like intestinal parasitic infection and malaria. The availability of local information on the magnitude and related risk factors has a major role in the management and control of anemia in pregnancy. Thus, the aim of this study was to investigate the fundamental hematological profiles of pregnant women in comparison with non pregnant women and to determine prevalence and associated risk factors of anemia in pregnant women at DURH.

1.3 Rationale of the study

Understanding of hematological changes accompanied by normal pregnancy was important to set appropriate strategy to control pregnancy complications. This will help the community and the government by conducting complete blood count for proper management of antenatal women. It also imposes to establish local hematological reference value of pregnant women. In addition, this study provides information on the current prevalence and associated factors of anemia among pregnant women attending ANC at DURH. The study will also serve as a reference for researchers who are interested to similar study.

2. Literature review

2.1.Previous study on Hematological profiles and anemia

A cross sectional study conducted by Chaudhari SJ, *et al*. in 2015 at New Civil Hospital, Surat, compared hematological parameters between pregnant women in third trimester and non pregnant women. The study revealed that pregnant women in third trimester exhibited statistically significant lower value of mean Hb value (11.4± 3.01) and mean RBC 3.5 ± 0.61 compared to non pregnant women (Hb12.7 ±0.94,RBC 4.48±1.2). Mean WBC and mean ESR was higher in third trimester pregnant women in comparison to non pregnant. They conclude that altered hematological indices are seen during third trimester of normal pregnancy in comparison to non pregnant women [36].

Cross sectional prospective study conducted in Nigeria in 2010 revealed that decrease hemoglobin concentration as well as slightly raised Neutrophil and Lymphocyte count that show significant difference in pregnant subjects in comparison with non-pregnant subjects. However, HCT and Hb were found to reduce significantly while WBC and Neutrophil increase slightly as trimester increased. There was no statistically significant difference in HCT, Eosinophil, Monocyte and Basophil between non-pregnant and pregnant subjects. Parity showed not to have influence on hematological parameters of pregnant women [37].

A similar study conducted in 2013 by Ichipi-Ifukor PC *et al*, reported the changes in hematological indices in normal pregnancy. They found that there was a significant decrease in the PCV (32.58±4.01) %, Hb (10.00±1.28)g/dl, granulocyte (59.91± 7.71) and PLT 202.177± 48.75 whereas lymphocytes increased (29.10 ±8.2) significantly in pregnant women when compared to the control (PCV 37.07±3.19 %, Hb11.71±1.32 g/dl, granulocyte 64.78 ± 11.45, PLT 224.863 ±75.21, lymphocyte 23.4 ± 6.9). WBC showed no significant difference; there was an increased level compared to the control. The study concluded that pregnancy in women has the tendency to alter hematological indices [38].

In southwest Nigeria in 2011, a study conducted by Osonuga IO, *et al*. showed that pregnant women exhibited statistically significant lower value of PCV (31.72±4.30), monocyte (1.41±0.80) and lymphocyte (35.68±14.50) while WBC (7.29±3.00), eosinophil (10.35±4.30)

and ESR (31.46±8.90) were not significantly changed compared to non pregnant women (PCV 38.75±3.70, Monocyte 4.16±1.90, lymphocyte44.86±12.50, WBC 4.93±0.90, eosinophil 6.32±3.40 and ESR 11.07±4.70). There was no significant difference in all hematological parameters among the three trimesters. They concluded that healthy pregnancy may have effect on hematological parameters [34].

On the other hand, another study carried out in 2012 at Lagos, Nigeria by Akinbami *et al*. showed hematological profile of normal pregnant women, a statistically significant relationship was found to exist between PCV (32.07% ± 6.80%, 29.76% ± 5.21%, 33.04% ± 3.88%) and WBC ($7.31 \pm 2.38 \times 10^9$, $7.88 \pm 2.33 \times 1\ 0^9$, and $8.37 \pm 2.15 \times 1\ 0^9$) count from first to third trimester respectively. However, there was no statistically significant association between PLT ($231.50 \pm 79.10 \times 1\ 0^9$, $227.57 \pm 63 \times 1\ 0^9$, and $200.82 \pm 94.42 \times 10^9$) count and increase in gestational age [10].

A study conducted by Ifeanyi *et al*. (2014) in Nigeria significant changes was found in mean values of RBC, HCT, MCV, MCH, WBC, Lymphocytes, Monocytes, Eosinophil's in the pregnant women relative to non pregnant women. There were significant changes between the trimesters in most of the parameters showing carefulness with the clinical management of pregnant women at any stage of the pregnancy [39].

Longitudinal study conducted by Purohit *et al*. (2015) on hematological profile of normal pregnant women in western India, revealed that trimester variations were recorded for platelet count, RDW, PCV, TLC and DLC during pregnancy, while other parameters like RBC count, blood indices, and Hb concentration remain unaltered during pregnancy. Comparison of pregnant with non pregnant shows significant changes [40].

Study conducted in 2013 by Das S, *et al*. at Bankura Samilani Medical College, Bankura revealed that pregnant women exhibited statistically significant lower values of Hb, PCV, monocyte and lymphocyte while WBC, eosinophil and ESR were significantly elevated compared to non pregnant women [41].

Hospital based cross-sectional study was conducted by Belayneh *et al*. (2015) at Hawassa University Teaching and Referral Hospital to assess the prevalence of thrombocytopenia, and associated factors among pregnant women. The prevalence of thrombocytopenia is 13.50%,

dominantly with mild type. The mean (±SD) platelet count was 249.9× 10^9/L (±88.7). Thrombocytopenia is higher in pregnant women with anemia [42].

According to the WHO report in 2011, the global prevalence of anemia for pregnant women was 38.2% and for all women of reproductive age was 29.4%. Severe anemia is associated with substantially worse mortality and cognitive and functional outcomes [43].

In Ethiopia, anemia is the severe problem affecting pregnant mothers largely. According to Ethiopia Demographic and Health Survey (EDHS) report of 2011, 17% of Ethiopian women age 15-49 are anemic, with 13% mild anemia, 3% having moderate anemia, and 1% having severe anemia. A higher proportion of pregnant women are anemic 22% than women who are breastfeeding (19 %) and women who are neither pregnant nor breastfeeding 15 % [44].

Institutional based cross sectional study was conducted in Tikur Anbesa Specialized Hospital from November to March, 2013 on 395 pregnant mothers attending ANC. The overall prevalence of anemia was 21.3 %. Out of the anemic pregnant mothers, 80.95% were mildly anemic, 17.86 % were moderately anemic and 1.19 % were severely anemic. Factors like age (39-45 years), education status (illiterate), family size (greater than four), gestational age (third trimester), birth intervals (less than two years), history of blood loss, no ANC, multigravida, and multiparous were significantly associated with anemia [45].

One cross sectional study was conducted in Nekemte Referral Hospital from April–May, 2014 to determine prevalence and Associated Risk Factors of anemia in Western Ethiopia. A total of 286 pregnant women were involved in the study. The overall prevalence of maternal anemia was 13.3%. Of the anemic women, 72.20%, 25 % and 2.80% were mildly, moderately, and severely anemic, respectively. The prevalence of anemia was high; mild type anemia was dominant. Low family income, having low dietary level and body mass index, STH (soil transmitted helminthes) and HIV (human immunodeficiency virus) infection was significantly associated with anemia [46].

In Bisidimo Hospital, South East Ethiopia, across sectional study was conducted from March to June 2013 to determine the prevalence and associated factors of anemia among pregnant women. The overall prevalence of anemia was 27.9%. The mean Hb value was 11.4 ± 2.3 g/dl. Of the anemic pregnant women, 55%, 32.5% and 12.5% had mild, moderate and severe anemia,

respectively. Rural residents, intestinal parasitic infections and history of heavy menstrual cycle were predictor of anemia [47].

A cross sectional study conducted in 2014 at Mekelle town to assess prevalence of anemia and associated factors among pregnant women attending antenatal care shows 19.7% overall prevalence of anemia. Meal frequency less than two per day, low dietary diversity score, medium dietary diversity score, parity and meat consumption less than once per week were found to be factors affecting anemia in pregnant women [48].

A facility based cross-sectional study was conducted on 363 pregnant women attending ANC clinic in Wolayita Soddo, Otona Hospital from January to March 2014. Overall, the prevalence of anemia was 39.94%. Among anemic pregnant women, 30.34%had mild anemia, 60%had moderate anemia, and 9.66% had severe anemia. From a total of anemic pregnant women, 75.17%, 22.07% and 2.76% had normocytic normochromic, microcytic hypochromic and macrocytic anemia, respectively. Age 15-24 years, family size >5,multigravida, having low income, current clinical illness, intestinal parasitic infection, no history of contraceptive usage, being in third trimesters , history of excess menstrual bleeding and low body mass index were identified as independent predictors of anemia among pregnant women [49].

3. Objectives

3.1 General objective

- To assess hematological profiles of pregnant women in comparison with non pregnant women and to determine prevalence and associated risk factors of anemia in pregnant women at DURH from March to May 2016.

3.2 Specific objective

- To compare hematological profile of pregnant women by trimester
- To compare hematological profile of pregnant and non pregnant women
- To determine the prevalence and associated risk factors of anemia among pregnant women.
- To characterize anemia morphologically

4. Hypothesis

- Hematological profile of pregnant women's are expected to be different from non pregnant women**.**
- Anemia prevalence is expected to be higher in pregnant women.

5. Materials and Methods

5.1Study site

The study was conducted in DURH found in Gedeo zone, Southern Nations Nationalities and Peoples Region (SNNPR). Dilla is found in 360 km away from Addis Ababa, which is situated at 1123.47 sq.kms at 6^0 21'-6^0 24'north latitude and 38^0 17'-38^0 20' east longitude. It is found in kola agro ecological zone with an altitude of 1400km above sea level and annual temperature ranging from 22^0c -29^0c in the south west of Ethiopia [50]. The Hospital was established in 1976 E.C. It is the only Referral and Teaching Hospital in the south of southern region providing health care services for Gedeo zone administrative area and the residences around including from the boarder of the country. The University Hospital is staffed by specialists from different disciplines like surgery, obstetrics and gynecology, pediatrics, internal medicine, and other health practitioners with different qualities. The Referral Hospital also has academic and supportive staffs and students in practice. The Hospital is providing different types of health care services for patients, and serves as training center for students from different departments in degree and post graduate programs

5.2Study period

The study was carried out from March to May 2016.

5.3Study design

A comparative cross sectional study was conducted to assess the hematological profiles of pregnant women in comparison with healthy non pregnant women (as a control) at DURH.

5.4Population

5.4.1 Source population

The Source populations were pregnant and non pregnant women who visit DURH during the study period.

5.4.2 Study population

The study population was pregnant women who visit DURH for antenatal care and apparently healthy age matched non pregnant women (as a control) who visit DURH as a relative of patient, and from DURH staff and who fulfilled the inclusion criteria.

5.5 Inclusion and exclusion criteria

5.5.1 Inclusion criteria

- Pregnant and non pregnant women age greater than 18 years.
- Women who gave informed consent

5.5.2 Exclusion criteria

- Women with acute illness (active bleeding, acute febrile illness and diarrheal diseases)
- Known HIV infected women
- History of anemia
- Insufficient, clotted and hemolyzed sample
- Women greater than 40 years

5.6 Study variables

5.6.1 Dependent variable

- Hematological profiles
- Prevalence of anemia

5.6.2 Independent variable

- Age
- Residence
- Educational status
- Occupation
- Income
- Diet

- Trimester of pregnancy
- Presence of intestinal parasitic infection
- Presence of malaria

5.7 Measurement and data collection

5.7.1 Sample size determination and sampling method

The Hematological profile of pregnant women in comparison with non pregnant was not known from previous study in Ethiopia. To calculate the sample size two populations mean was used by assuming that the two populations mean difference of hemoglobin is 0.5g/dl [37].

$$n_1 = \left(1 + \frac{1}{r}\right)\frac{z\frac{\alpha}{2}\sigma^2}{d^2}, \text{ and } \quad n2 = rn_1$$

Where:

n_1= number of pregnant subject (comparison group)

n_2= number of non pregnant subject (control group)

r = is the ratio of the Population allocation of pregnant women to non pregnant group;

$r = \frac{n}{n1} = \frac{1}{1} = 1$

Level of confidence 95% (α=0.05)

Zα/2= Value of the standard normal distribution curve corresponding to the level of significance α = 1.96

d =mean difference of hemoglobin between pregnant and non pregnant women = 0.5

σ^2 = is pooled variance which is 1.65[37].

According to the formula the calculated sample size was 84 and by adding 10% for non response rate, the final sample size for this study was 93 for pregnant and 93 for non pregnant with total

sample size of 186 women's. 93 consecutive normal healthy pregnant women's were selected and 93 randomly selected age matched non pregnant women were used as a control.

5.7.2 Data collection procedure

5.7.2.1 Questionnaire

A structured, pretested and interviewer administered questionnaires were employed to obtain data on demographic and socio-economic variables, maternal characteristics and dietary habits. The questionnaire used in the study was in English and it was translated to Amharic. The questionnaire was filled by trained nurses [Annex III].

5.7.2.2 Blood Specimen collection and processing

Blood specimen was collected by disinfecting the phlebotomy site by swabbing the skin in small outward circles with 70% alcohol swab. A total of 3mL venous blood sample was drawn from each participant into Ethylene diamine tetra acetic acid (EDTA) tube under sterile condition for complete blood count and peripheral blood smear [Annex IV].

Hematological analyses were conducted by the principal investigator using CELL DYN 1800 (Abott Laboratories Diagnostics Division, USA). The Hematological parameters measured includes: WBC, RBC, Hb, HCT, MCV, MCH, MCHC, WBC Differential count, RDW, and PLT. In this study, anemia was defined as when the amount of Hb is <11 g/dl for pregnant women. Also mild, moderate and severe anemia was defined as when the Hb amount was between 10-10.9 g/dl, 7-9.9 g/dl and less than 7 g/dl, respectively [11]. Anemia was morphologically characterized as normocytic, microcytic and macrocytic based on red cell size and normochromic and hypochromic based on their hemoglobin content (central pallor area).

A drop of blood was placed on a clean slide and, thick and thin smears were prepared on separate slide. After being air- dried, labeled with identification number, the thin smears were fixed with absolute methanol, and the smear was stained with Geimsa staining solution. Using light microscope, examination of the thick and thin smear were done with high power magnification (40 x objectives) and oil immersion (100 x objectives) to investigate malaria parasite and type of anemia based on the morphology of red blood cell respectively [Annex VI]. The blood smears were examined by two senior laboratory technologists and the principal investigator separately.

5.7.2.3 Stool specimen collection and processing for wet smear

Participants were requested to give fresh stool sample for parasitological examination of intestinal parasites. Stool sample was collected by using a clean and labeled container from each study participant. A drop of normal saline was put on the cleaned microscopic slides; a small amount of stool specimen with a wooden applicator stick was taken and mixed with saline and cover with cover glass. Two senior laboratory technologists were examined the collected and prepared stool samples with in 30 minute using 10x objective and 40x objectives for detection of helminthes eggs, larvae and cysts of protozoan parasites [Annex VII].

5.7.3 Principle of Cell-Dyn 1800 Hematology Analyzer

The Cell-Dyn 1800 Hematology Analyzer performs a Complete Blood Count (CBC)

The CELDYN 1800 uses two independent measurement methods; they are:

- Electrical impedance method for determining WBC, RBC, and PLT data
- Modified Methemoglobin Method for determining HGB

During each instrument cycle, the sample is aspirated, diluted, and mixed before each parameter is measured.

Electrical impedance is used to count the white blood cells, red blood cells, and platelets as they pass through an aperture. As each cell is drawn through the aperture, a change in electrical resistance occurs generating a voltage pulse. The number of pulses during a cycle corresponds to the number of cells counted. The amplitude of each pulse is directly proportional to the cell volume.

Lyse reagent is added to the diluted sample and used to count the white blood cells. After the white blood cells have been counted and sized, the remainder of the lysed dilution is transferred to the Hb Flow Cell to measure Hemoglobin concentration. The Cell-Dyn uses electronic sizing to determine a three part automated differential. The percentage and absolute counts are determined for lymphocytes, neutrophil, and mid-size population of monocytes, basophils, eosinophils, blasts, and other immature cells.

5.7.4 Principle of Geimsa stains

Geimsa stain is Romanowsky stains which contain eosin Y which is an acidic anionic dye and azure B and other thiazine dyes (derived from the oxidation or polychroming of methylene blue) which are basic cationic dyes. When Romanowsky dye is diluted with buffered water, ionization occurs. Acidic dyes unites with the basic components of the cell (cytoplasm) and hence the cytoplasm is eosinophilic (acidic). Conversely, basic stains are attracted to and combine with the acidic parts of the cell (nucleic acid and nucleoproteins of the nucleus) and hence these structures are called basophilic. Other structures stained by combination of the two are neutrophilic.

5.8 Data quality control

5.8.1 Pre-analytical

To assure the quality of the data, training was given to the data collectors and the questionnaire was pretested. Standard operating procedures were strictly followed during specimen collection and laboratory procedures.

5.8.2 Analytical

Commercially available low, normal and high quality control reagents were used to check the reliability (accuracy and precision) of the data generated by the hematology analyzer and SOPs were strictly followed in each laboratory procedures. The qualities of Geimsa staining solution were checked with prepared known malaria positive and negative control slides.

5.8.3 Post analytical

The accuracy and completeness of the collected data were checked every day by the principal investigator. Data was cleaned, coded and entered correctly.

5.9 Data analysis and interpretation

All the data collected from the laboratory investigation and questionnaire were analyzed using SPSS version 20 software. Dependent variable percentage, mean and standard deviation were calculated. Binary logistic regression was used to determine the association of anemia and demographic and clinical variables. Multiple logistic regressions were used to control the

confounding factors. Differences in mean values were determined by independent t test and one way ANOVA for pregnant women and non pregnant women (controls). P-values < 0.05 were taken as statistically significant.

5.10 Ethical consideration

The study was conducted after obtaining ethical clearance from Department Ethical Review Committee of Medical Laboratory Science, Addis Ababa University. Support letter was obtained from the Department of Medical Laboratory Science. Official permission was also obtained from DURH. The data were collected after the written informed consent obtained from the participant. To maintain confidentiality all the information obtained from the study participants were uniquely identified and coded. Any positive results were timely reported to the clinicians for appropriate intervention. In addition, the clinical specimens collected during the study period were used only for the stated objectives.

5.11 Dissemination of the result

The finding of this thesis will be submitted to Department of Medical Laboratory Sciences, Addis Ababa University. It will be serving as a reference in the library. In addition a copy of this material will be given to DURH. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshops and seminars.

6. Result

6.1. Socio demographic data of the study population

The study population comprised 110 pregnant women and 110 non pregnant women's (control group). The mean age of controls and pregnant women's were 24.9±4.8 and 24.57±4.5 years old, respectively. Majority of the study participants 37(33.6%) control and 47(42.7%) pregnant women were in age group 21-25 years. The difference between pregnant and non pregnant women in terms of age distribution was not significant (p=0.682). Most of the respondents 78(70.9%) control and 83(75.5%) pregnant women were urban resident, and there was no statistical significant difference in terms of residence (p=0.543). Majority of pregnant women 39.1% had primary education and 35.5% control had higher educational level. Analysis of educational status of the study participant showed significant difference at various educational level between pregnant and non pregnant (p=0.005). Regarding occupational status the majority of the respondent 66(60%) pregnant and 49(44.5%) controls were house wives and there was statistical significant difference between pregnant and control groups (p=.001). On the other hand, 30% pregnant women and 38.2% control had 501- 850 birr monthly income. There was no statistical significant difference in terms of monthly income (p= .495) (Table 1).

Concerning dietary habit, most of pregnant 64(58.2%) and non pregnant 75(68.2%) had the habit of eating animal food products weekly. Majority of pregnant women 46(41.8%) and 44(40%) had the habit of eating green vegetables and fruits every other day where as 69(62.7%) and 41(37.3%) control had the habit weekly. Regarding drinking tea/coffee immediately after meal, most of pregnant women 63(57.3%) had the habit than the control 55(50%). Significant difference in dietary habit was observed on eating green vegetables between pregnant and control group (Table 1).

Table 1: Socio demographic characteristics and dietary habit of study participants at DURH from March to May 2016, Dilla; Ethiopia

Variables		Non pregnant women (control) No. (%)	Pregnant women No. (%)	X^2	P-value
	≤20	30(27.3)	28(25.5)		
	21-25	37(33.6)	47(42.7)		
	26-30	28(25.5)	24(21.8)		
	31-35	13(11.8)	10(9.1)		
	>35	2(1.8)	1(0.9)	2.292	0.682
Age (years)	Mean ±SD	24.9±4.8	24.57±4.5	F=0.389	0.533
	Urban	78(70.9)	83(75.5)		
Residence	Rural	32(26.1)	27(24.5)	0.371	0.543
	Illiterate	14(12.7)	9(8.2)		
	Primary	27(24.5)	43(39.1)		
Educational	Secondary	30(27.3)	39(35.5)		
status	college	39(35.5)	19(17.3)	12.815	0.005
	Private	7(6.4)	15(13.6)		
	Governmental	31(28.2)	22(20)		
occupational	House wife	49(44.5)	66(60)		
status	Other	23(20.9)	7(6.4)	15.4	0.001
	≤ 500	16(14.5)	22(20)		
	501-850	42(38.2)	33(30)		
monthly	851-1200	21(19.1)	25(22.7)		
income	> 1200	31(28.2)	30(27.3)	2.392	0.495
Frequency	I don't eat	3(2.7)	3(2.7)		
of eating	Daily	0	1(0.9)		
animal food	Every other day	1(0.9)	7(6.4)		
products	Weekly	75(68.2)	64(58.2)		
	Monthly	31(28.2)	35(31.8)	6.613	0.158
Frequency	I don't eat	1(0.9)	1(0.9)		
of eating	Daily	4(3.6)	18(16.4)		
green	Every other day	35(31.8)	46(41.8)		
vegetables	Weekly	69(62.7)	43(39.1)		
	Monthly	1(0.9)	2(1.8)	16.772	0.002
Frequency	I don't eat	1(0.9)	3(2.7)		
of eating	Daily	23(20.9)	20(18.2)		
fruits	Every other day	41(37.3)	44(40)		
	Weekly	45(40.9)	42(38.2)		
	Monthly	0	1(0.9)	2.419	0.659
Drinking	Yes	55(50)	63(57.3)		
tea/coffee					
after meal	No	55(50)	47(42.7)	0.896	0.34

6.2 Hematological profile of the study population

The overall mean± SD hematological profiles for pregnant women were as follows: WBC 7.81± 2.12× 10^9/L, RBC 4.11±0.37× 10^{12}/L, Hb 11.9 ± 1.00 g/dl, HCT 37.72 ± 3.07%, MCV 91.74 ± 3.62fl, MCH 29.08 ± 1.59 pg, MCHC 31.7 ± 1.11%, RDW 13.67±0.77%, PLT 246.87±80.38× 10^9/L, Lymphocyte 27.08±7.04%, Neutrophils 66.8±8.21% and MID 6.04±2.63%. The result of this study showed that there was a statistically significant decrease in total RBC, Hb, HCT, MCHC, Lymphocyte, MID and PLT counts in pregnant women when compared with non pregnant. WBC, neutrophils and MCH were significantly higher in pregnant women as compared to non pregnant (control). MCV and RDW showed no significant difference (Table 2).

Table 2: Comparison of hematological profile of pregnant and non pregnant women in DURH from March to May 2016, Dilla; Ethiopia

Parameters	**pregnant women (n=110)**	**Non pregnant women (control) (n=110)**	**P value**
RBC ×10^{12}/L	4.11±0.37	4.45±0.33	<0.001*****
Hb(g/dl)	11.9± 1.00	13.18 ± 1.00	<0.001*****
HCT (%)	37.72 ± 3.07	40.77 ± 2.87	<0.001*****
MCV(fl)	91.74 ± 3.62)	91.36 ± 3.27	0.415
MCH(pg)	29.08 ± 1.59	29.5 ± 1.11	0.023*****
MCHC (%)	31.7±1.11	32.3 ± 0.97	<0.001*****
RDW (%)	13.67±0.77	13.55±0.86	0.246
WBC× (109/L)	7.81± 2.12	6.3±1.88	<0.001*****
Lymphocyte (%)	27.08±7.04	38.49±10.5	<0.001*****
MID (%)	6.04±2.63	7.54±2.53	<0.001*****
Neutrophil (%)	66.8±8.21)	54.05±11.41	<0.001*****
PLT× (109/L)	246.87±80.38	291.34± 70.68	<0.001*****

* P< 0.05: statistically significant

As shown in table 3, when hematological parameters were analyzed by trimester, there was a significant deference in RBC, Hb, HCT and RDW. The mean RBC count (3.99±0.33), Hb (11.66±0.95) and HCT (36.74±2.99) in the second trimester pregnant women was significantly

decreased (P value = 0.010, 0.006 and 0.003 for RBC, Hb and HCT, respectively) compared to first trimester pregnant women (RBC 4.23±0.41, Hb 12.35±0.97 and HCT 38.9±3.01). Mean RDW value in the three trimesters were 13.4±0.64%, 13.73±0.67% and 13.98±0.96% with a statistically significant difference between 1st and 3rd trimester pregnancy (P=0.011). The mean WBC counts shows slight increment from 1st to 3rd trimester but the difference was not statistically significant. The mean red cell indices (MCV, MCH and MCHC), PLT and differential WBC counts in the three trimesters were not statistically different.

Table 3: Comparison of hematological profile of pregnant women based on trimester in DURH from March to May 2016, Dilla; Ethiopia

Parameter	**1st trimester**	**2nd trimester**	**3rd trimester**	**P value**		
				1st&2nd	**1st&3rd**	**2nd&3rd**
WBC (10^9/L)	7.43±1.85	7.84±2.42	8.55±1.79	0.647	0.098	0.363
Lymphocyte (%)	28.14±6.72	27.42 ±7.65	24.82±6.04	0.882	0.156	0.296
Mid (%)	5.69 ±2.11	6.33±3.15	6.05±2.32	0.516	0.860	0.906
Neutrophils (%)	66.15± 7.61	66.12±9.17	69.11±7.05	1	0.340	0.311
RBC(10^{12}/L)	4.23±0.41	3.99±0.33	4.11±0.34	0.010*	0.400	0.419
Hb(g/dl)	12.35±0.97	11.68±0.95	11.93±0.99	0.006*	0.218	0.561
HCT (%)	38.9±3.01	36.74±2.99	37.72±3.06	0.003*	0.241	0.410
MCV(fl)	91.98±4.29	92.03±2.76	90.82±3.84	0.998	0.421	0.371
MCH(pg)	29.13±1.81	29.25±1.16	28.72±1.90	0.943	0.574	0.384
MCHC (%)	31.72±1.01	31.76±1.06	31.6±1.37	0.979	0.909	0.821
RDW (%)	13.42±0.65	13.72±0.67	13.98±0.96	0.170	0.011*	0.324
Platelet (10^9/L)	262.77±85.73	244.09±72.26	227.2±84.19	0.533	0.197	0.673

* $p<0.05$: statistically significant

6.3Prevalence of anemia

The overall prevalence of anemia using a cut off level of hemoglobin value < 11g/dl for pregnant women and < 12g/dl for non pregnant women was 15.5% (17/110) and 10% (11/110) respectively. Of the anemic pregnant women, 94.1% and 5.9% were mildly and moderately anemic, respectively and no sever anemia observed (Figure1).

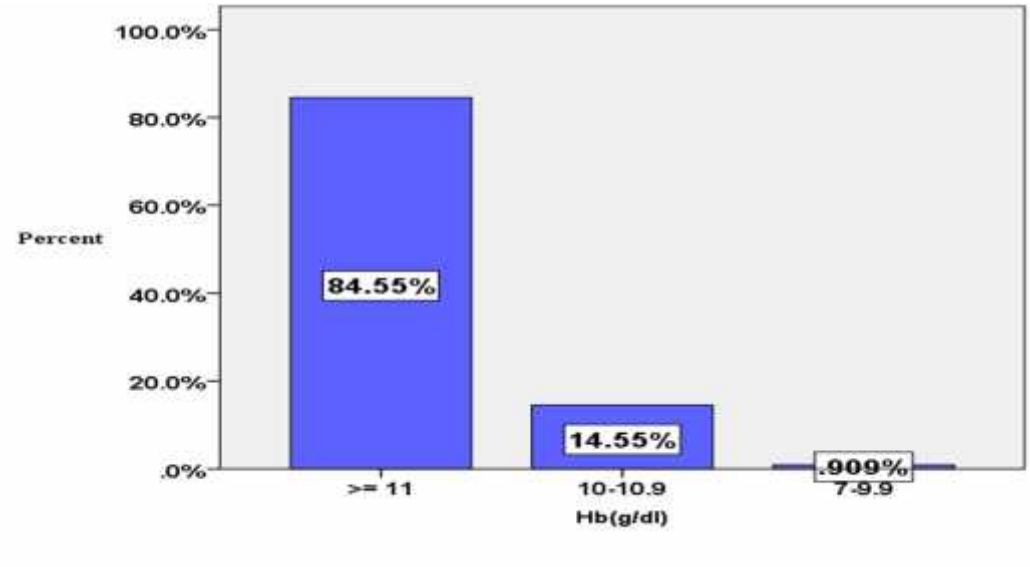

Figure 1: Prevalence and degree of anemia among pregnant mothers attending ANC at DURH from March to May, 2016, Dilla, Ethiopia.

When anemia was characterized based on red cell morphology, 12(70.6%) anemic pregnant women had normocytic normochromic, 3(17.6%) microcytic hypochromic and 2(11.8%) macrocytic normochromic type of anemia (Fig 2).

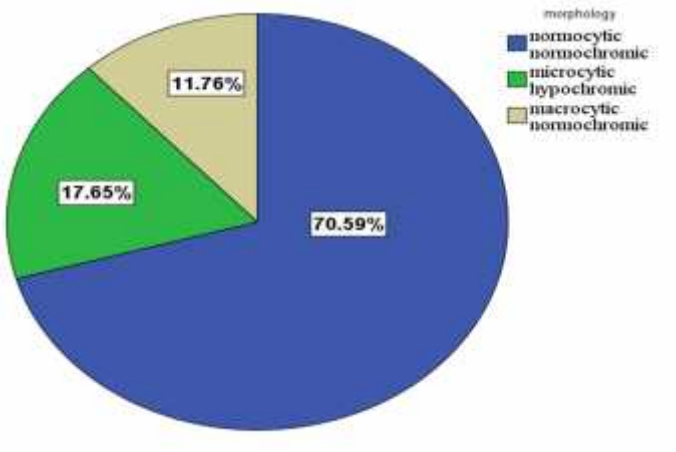

Figure 2: Morphologic types of anemic pregnant women attending ANC at DURH from March to May 2016, Dilla; Ethiopia.

Out of the anemic, about 52.9%, 23.5%, 11.8% and 11.8% of the pregnant women were in the age range of 21-25, 26-30, 30-35 and ≤ 20 years, respectively. Nearly half of the women with anemia were house wives (47.1%), and had been educated at secondary school (41.2%). Out of the anemic pregnant women, (82.4%) lived in urban areas and the rest 17.6% were rural dwellers Of the anemic pregnant women, the prevalence of anemia was higher in the second trimester 64.7% and third trimester 29.4% pregnancy than first trimester 5.9% pregnancy. Among the anemic pregnant women, 64.7%, 17.6%, 5.9%, 5.9%, and 5.9% were with no ova or parasite, *A. lumbricoides, H. worm, E. histolytica* and *G.lamblia*, respectively and 5.9% with malaria. Majority of anemic pregnant women had the habit of eating animal food (11/17), green vegetables (9/17) and fruits weekly (9/17), and drink tea or coffee (12/17) immediately after meal (Table 4).

In bivariate analysis attaining primary school (COR=7.321 (95% CI=1.274-42.067) and being in second trimester pregnancy (COR=0.195(95%CI=0.04-0.95) were significantly associated with maternal anemia. Variables that show P value less than 0.2 in bivariate analysis were taken to multivariate analysis. But in multivariate logistic regression analysis only primary educational level was significantly associated with anemia in the study population. Pregnant women who had primary educational level were 6.82 times more likely to be affected by anemia as compared with higher educational level (college) (AOR= 6.82(95% CI=1.149, 40.527) (Table 4).

Table 4: Association of risk factors with anemia among pregnant women attending antenatal care in DURH from March to May, 2016, Dilla, Ethiopia

Characteristics		Anemia status		COR(95%CI	AOR(95%CI)	P Value
		Anemic N (%)	Non anemic N (%)			
	≤20	2(11.8)	26(20.8)	1		
	21-25	9(52.9)	38(40.9)	0.325(0.065, 1.627)		
	26-30	4(23.5)	20(21.5)	0.385(0.064, 2.314)		
	31-35	2(11.8)	8(8.6)	0.208(0.037, 2.548)		
Age(years)	>35	0	1(1.1)	0		
	Urban	14(82.4)	69(74.2)	1		
Residence	Rural	3(17.6)	24(25.8)	1.623(0.429, 6.141)		
	Illiterate	3(17.6)	6(6.5)	0.714(0.128, 3.995)	0.696(0.114, 4.236)	0.694
	Primary	2(11.8)	41(44.1)	**7.321(1.274,42.067)***	**6.82(1.149, 40.527)**	**0.035***
	Secondary	7(41.2)	32(34.4)	1.633(0.441, 6.040)	1.738(0.452, 6.685)	0.422
Educational status	college	5(29.4)	14(15.1)	1	1	
	Private	1(5.9)	14(15.1)	1		
	Governmental	6(35.3)	16(17.2)	0.190(0.02, 1.78)		
	House wife	8(47.1)	58(62.4)	0.518(0.060, 4.487)		
occupational status	Other	2(11.8)	5(5.4)	0.179(0.013, 2.425)		
	≤ 500	4(23.5)	18(19.4)	1		
	501-850	5(29.4	28(30.1)	1.244(0.294, 5.263)		
	851-1200	2(11.8)	23(24.7)	2.556(0.42, 15.553)		
monthly income	> 1200	6(35.3)	24(25.8)	0.889(0.218, 3.623)		
	I don't eat	1(5.9)	2(2.2)	0.333(0.025, 4.401)		
	daily	0	1(1.1)	0.00(.00)		
	every other day	0	7(7.5)	0.00(.00)		
Frequency of eating animal food products	Weekly	11(64.7)	53(57)	0.803(0.255, 2.531)		
	Monthly	5(29.4)	30(32.3)	1		
	I don't eat	1(5.9)	0	0.000(0.000)		
	Daily	0	18(19.4)	0.000(0.000)		
	every other day	7(41.2)	39(41.9)	0.000(0.000)		
Frequency of eating green vegetables	Weekly	9(52.9)	34(36.6)	0.000(0.000)		
	Monthly	0	2(2.2)	1		
	I don't eat	0	3(3.2)	1.00(0.00)		
	Daily	2(11.8)	18(19.4)	0.000(0.000)		
	every other day	6(35.3)	38(40.9)	0.000(0.000)		
	Weekly	9(52.9)	33(35.5)	0.000(0.000)		
Frequency of eating fruits	Monthly	0	1(1.1)	1		
Drinking tea /coffee after meal	Yes	12(70.6)	51(54.8)	1		
	No	5(29.4)	42(45.2)	1.976 (0.645, 6.06)		
	First	2(5.9)	37(39.8)	1		
	Second	10(64.7)	36(38.7)	**0.195(0.04, 0.95)**	0.209(0.041,1.062)	0.059
Trimester	Third	5(29.4)	20(21.5)	0.216(0.038, 1.217)	0.24 (0.04, 1.428)	0.117
	Negative	16(94.1)	92(98.9)	1		
malaria	Positive	1(5.9)	1(1.1)	0.174(0.010,2.924)		
	no ova/parasite	11(64.7)	76(81.7)	1	1	
	A. Lumbricoides	3(17.6)	7(7.5)	0.338(0.076, 1.503)		
	T. trichuria	0	2(2.2)	2.34E(0)		
	H. worm	1(5.9)	1(1.1)	0.145(0.008, 2.485)		
	E. histolytica	1(5.9)	2(2.2)	0.289(0.024, 3.465)		
Intestinal parasite	*G.lamblia*	1(5.9)	5(5.4)	0.724(0.077, 6.786)		

* P < 0.05: statistically significant, 1 =Reference group, COR= Crude odd ration, AOR= Adjusted odd ration 95%C.I=95% confidence interval

7. Discussion

This study was carried out at DURH during the period from March to May 2016 to assess the hematological parameters of pregnant women. It revealed that mean Hb (11.9±1.0), HCT (37.72±3.07), RBCs count (4.11±0.37) and MCHC (31.7±1.11) of pregnant women were significantly decreased ($p < 0.05$) compared with control group (Hb 13.18±1.0, HCT 40.77±2.87, RBC 4.45±0.33 and 32.3±0.97). The values of MCV were not different where as MCH value showed significant increase in pregnant women when compared with non pregnant. These results agreed partly with the result of the study by Verma *et al* in India which showed that during pregnancy RBC count, Hb concentration and HCT value were lower, and MCH value were higher [20] and Elgari (2013) reported that there were significant decrease in RBCs count, Hb, HCT and MCHC at pregnancy period when compared with control group [51]. Also agreed with a study in Nigeria by Ichipi-Ifukor *et al* who found that significantly decreased PCV (32.58±4.01) % and Hb (10.00±1.28)g/dl in pregnant women [38].

There is statistically significant decrease in the value of Hb 11.66±0.95, HCT 36.69±2.98, and RBC (3.99±0.33) in second trimester pregnancy compared to first trimester (Hb 12.39±0.94, HCT 39.02±2.96 and RBC 4.24±0.42) and begin to rise in the third trimester but not statistically significant. The study is in accordance with a study conducted by James *et al* (Hb 12.73 ± 1.14, 11.41 ± 1.16 and 11.67 ± 1.18, and HCT 37.05 ± 2.96, 33.12 ± 3.00 and 34.03 ± 2.97 for 1^{st}, 2^{nd} and 3^{rd} trimester respectively) [52] and Akinbami *et al* (HCT 32.07 ±6.80, 29.76 ±5.21, and 33.04 ±3.88) [10]. The study contradicts with Osonuga *et al* [34] and Ifeanyi *et al* [39] in Nigeria which showed Hb and HCT value were lowest in first trimester, rise in second trimester and drops in third trimester. The main cause of hemoglobin reduction may be due to physiological changes associated with pregnancy in which a reduction in hemoglobin concentration result from dilution due to the plasma volume expands more than the erythrocyte volume [11].

The result of this study showed no statistically significant difference in RDW value between pregnant (13.67±0.77%) and control group (13.55±0.86%)**,** however when RDW values were compared in trimester (1^{st} 13.4±0.64%, 2^{nd} 13.73±0.67% and 3^{rd} 13.98±0.96%) slightly increased value was observed with a significant deference in the first and third trimester. Our finding is

consistent with findings of Purohit *et al* (14.07± 1.01, 15.07±1.01 and 18.9±3.85 in first, second and third trimester respectively) [40].

In the present study, there was significant increase in mean of white blood cell (7.81±2.12) and neutrophils (66.8±8.21) count of pregnant women (P <0.001) compared to non pregnant women (WBC 6.31±1.88 and neutrophil 54.05±11.41). Similar findings were reported by Pughikumo *et al*, which showed significant increase in WBC (6.86±1.54) count in pregnant women than non pregnant women (5.8± 1.41)[19]. Okpokam *et al* also reported significantly higher value of WBC (8.3± 5.94) in pregnant women than non pregnant women (6.2± 2.03) [37]. Leukocytosis occurring during pregnancy may be due to physiological stress induced by the pregnant state or as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, immunosuppression and immunomodulation in the presence of a strong antimicrobial immunity [53, 54].

Lymphocyte proliferation in response to a variety of agents was found to be impaired in pregnancy, suggesting that there is an immunosuppressant factor present in the serum [11]. There was a significant decrease in Lymphocyte and MID white blood cell count in pregnant women when compared with non pregnant women. This agrees with previous work by Verma *et al* that reported decreased lymphocytes (26.16 ±4.629), eosinophil (4.07±2.12), basophil (0.53±0.5) and monocyte (3.06±1.205) in pregnant women than control (29±3.279, 5.8±1.658, 0.640±0.49, and 3.86±1.08 for lymphocyte, eosinophil, basophil and monocyte respectively) [20].

A gradual significant decrease in PLT count was observed in pregnant women (246.87±80.38) when compared with non pregnant (291.34±70.67). This findings also supported by studies done in Nigeria by Ichipi-Ifukor *et al*(PLT 202.17±48.75 and 224.8±75.2) [38] and Ifeanyi *et al*(PLT 122±3.4 and 198.5± 5.6 for pregnant and non pregnant women respectively)[39]. Akinbami *et al* Purohit *et al* and Elgari also reported lower platelet count in pregnant women [10, 37, and 51]. Our study also reported slightly decreased platelet count between trimesters; however the deference was not statistically significant. The reduction of PLT count in pregnant women may be due to dilution effects and accelerated destruction of PLTs passing over the often scarred and damaged trophoblast surface of the placenta [10].

The prevalence of anemia obtained in this study was 15.5%, based on WHO criterion for the diagnosis of anemia in pregnancy, i.e. hemoglobin <11.0 g/dl [11]. This result is consistent with cross sectional study carried out in Tikur Anbesa Specialized Hospital (21.3%) [45], Mekele town (19.7%) [48], Gondar Teaching Hospital (16.6%) [55], Hawassa (15.1%) [56], Azezo Health Center (21.6%) [57].

The prevalence of anemia in this study is lower than a study conducted in Western Ethiopia (29%) [46], South-East Ethiopia (27.9%) [47], Southern Ethiopia Wolayita Sodo (39.9%) [49], Northern Ethiopia (36.1%) [58], West Arsi zone (36.6%) [59], Arba Minch (32.8%) [60], Gilgel Gibe Dama area (53.9%) [61], Boditi Health Center (61.6) [62], Nekemt Health Center (52%) [63].

The low prevalence of anemia in the current study might be due to differences in sample size geographical variation, and difference in socioeconomic status. In addition, lower prevalence can be attributed to continuing improvement of life style and living standards and administration of iron supplement in health centers which is helpful in combating anemia during pregnancy.

In this study, the majority of anemic cases 94.1 %(16/17) were of the mild type (Hb 10.0-10.9g/d1) followed by 5.9% (1/17) cases of moderate anemia (7-9.9g/dl). This result is some what lower than a study conducted in Wolayita Soddo, Azezo and Gilgel Gibe Dam area, southwestern Ethiopia where moderate anemia accounted for 60%, 46% and 42.1% respectively [49, 57 and 61]. This inconsistency may be because of the strengthened health education given on risk factors and prevention of anemia and interventions given at health institutions during ANC follow up in an attempt to reduce the prevalence and severity of anemia among pregnant mothers. In addition, it might because of time and place difference between the present study and the study conducted in Wolayita Soddo, Gilgel Gibe Dam area, southwestern Ethiopia and Azezo.

Of the total anemic pregnant women, 70.6% had normocytic normochromic anemia followed by microcytic hypochromic type which is in agreement with a report from Gondar teaching hospital, and Turkey where normocytic normochromic anemia accounted for 76% and 56.5% respectively [55, 64]. Higher percent of normocytic normochromic anemia in this study may be due to chronic disease and iron deficiency. Anemia in pregnancy is related to different socio-

demographic factors [30]. In different studies, age, residence, educational status, economic positions have been found to be significantly associated with anemia during pregnancy [44, 47, and 65]. This study assessed socio-demographic variables associated with anemia but only educational level (primary) had shown statistically significant association with anemia (AOR=6.28). This high prevalence of anemia in these study participants might be due to inadequate knowledge on factors causing anemia and on how to prevent the risk factors. The findings of the present study partly similar with studies from Tikur Anbesa Specialized Hospital which showed a statistical significant association of anemia with educational status (illiterate (AOR=2.12) [45]and Azezo Health Center which showed 8.8 higher for illiterate pregnant women as compared to those women with > 12 educational level [57].

One of the major contributory factors for anemia in developing countries is consumption of plant based food containing insufficient iron, especially insufficient available hem iron from meat [66]. Meat is a good source of high quality protein, iron and zinc and of all the B-vitamins except folic acid. Iron absorption is enhanced when consumed with foods high in vitamin C such as orange juice but substances in coffee and tea inhibit iron absorption [67]. This study has tried to assess different dietary risk factors associated with anemia. Eating animal food, green leafy vegetables, taking fruit and drinking tea/coffee after meal did not show significant association with anemia which may be due to no difference in eating habit among the study participants.

In many developing countries family planning is rarely practiced and many un-planned pregnancies take place while mothers are still breast feeding. This exposes the pregnant mother to extraordinary stress that affects her nutritional status and result in depletion of the micronutrient stores of the mother, to the extent that she becomes anemic from first trimester in the next pregnancy. The daily requirements for iron as well as folate are greater for a woman in the last two trimesters of pregnancy. This need cannot be met by diet alone, but is derived from at least partly from maternal reserves. When iron reserves are already low, due to malnutrition and or frequent pregnancies, anemia results and progresses to become severe in the second and third trimesters. However, this study failed to observe relationship between prevalence of anemia and gestational age, but majority of pregnancy related anemia cases were recorded in second (64.7%) and third (29.4%) trimesters of pregnancy. The same result was observed in a study conducted in Azezo, Gondar town [57].

Soil transmitted helminthes; particularly *hookworm* and *Trichuria* contribute to anemia in pregnancy leading to secondary iron deficiency anemia [12]. In this study however, no significant helminthic infections were identified and no significant relationship between those with helminthic infestation and the presence of anemia in pregnancy [Table 4]. The low level of helminthic infections may be because it was in done in an urban set up which is likely to have proper sanitation and clean drinking water as opposed to a rural set up. A significant number were also had formal education of secondary level and above and thus are able to observe good hygiene practices. Also, the stool sample was only taken once thus making it difficult to ascertain whether helminthes were a contributory factor. Repeat samples should be done in future to take into consideration the ova maturation cycle of helminthes.

Malaria, also known to be a major cause of anemia in pregnancy [32] was not found to be significant factors in this study as only two women were found to have a positive blood slide for malaria parasite [Table 4]. This may be due to season of the study being done.

8. Strength and Limitation of the study

8.1 Strength

- This study have control group

8.2 Limitation

- This study lacks concentration technique to detect intestinal parasites
- This study subjects were not examined for HIV, chronic disease, and etc.
- There may be a social desirability bias for monthly income and dietary information. This may under estimate the association between the variable and anemia among the pregnant women.

9. Conclusion and Recommendation

9.1 Conclusion

This study showed that statistical significant difference in RBCs, Hb, HCT, MCH, MCHC, PLT, TWBC and differential count between pregnant and non pregnant women ($p<0.05$) at DURH. However, there was no significant difference in MCV and RDW at p- value > 0.05.It also revealed, significant difference in Hb, HCT, RBCs and RDW between trimesters ($p< 0.05$) of pregnancy.

The overall prevalence of anemia in pregnant women in this study was 15.5%. In addition, in this study the majority of anemic pregnant women had mild type of anemia, which might be due to the undergoing strategy concerning primary health care in Ethiopia, seems well planned and practiced. Morphologically, normocytic normochromic type of anemia predominate microcytic hypochromic type of anemia followed by macrocytic normochromic. The present study indicated that there was a significant association between anemia and educational level (primary).

9.2 Recommendation

- Routine hematological tests particularly RBCs, Hb, PLT, WBCs and differential count should be done for pregnant women. So that pregnancy complications could be detected and managed.
- Statistical baseline data of hematological status of Ethiopian pregnant women is highly needed.
- In Ethiopia, data concerning hematological profile of pregnant women are few so future longitudinal studies are needed to help health authorities to implement polices to improve health status of pregnant women.
- Strength health care seeking behavior of women to ensure early diagnosis and management of anemia, and other medical conditions during pregnancy period.

10. References

1. Shinton NK. Desk reference for Hematology. 2nd ed. *CRC Press.* 2008; 377- 400.
2. Ashavaid TF, Todur SP, Dherai AJ. Establishment of reference intervals in Indian population. *Ind. J. Clin.Biochem*. 2005; 20(2):110–118.
3. Cunningham F, Leveno K, Haulth J, Touse D, Spong C. Williams textbook of obstetrics 23 ed. *McGraw Hill professional publishing*. 2010; p. 121.
4. Hopkins J. Manual of Gynecology and Obstetrics 4th edition. *Lippincott Williams and Wilkins*. 2012; p. 438.
5. Townsley DM. Hematologic Complications of Pregnancy. *Semin Hematol*. 2013; 50(3): 222–231.
6. Saleh MAD, Farhan AA, Alwan MH. Evaluation of physiological effect of pregnancy on some Immunological parameters for pregnant women. *IOSR J Den Med Sci*. 2015; 14(6): 35-41.
7. Haq IU, Ahmed MA, Anwar B, Saleem MA. Hematological Profile of healthy pregnant females in association with parity. *J Rawalpindi Med College*. 2013; 17(1): 98-100.
8. Salhan S, Tripathi V, Singh R, Gaikwad HS. Evaluation of Hematological Parameters in Partial Exchange and Packed Cell Transfusion in Treatment of Severe Anemia in Pregnancy. *Anemia*. 2012; 7.
9. Heidemann BH, McClure JH. Changes in maternal physiology during pregnancy. *British J Anesthesia*. 2003; 3(3):65-68.
10. Akinbami AA, Ajibola SO, Rabiu KA, Adewunmi AA, Dosunmu AO, Adediran A, et al. Hematological profile of normal pregnant women in Lagos, Nigeria. *Inter J Women"s Health*. 2013; 5:227-232.
11. Pavord S, Hunt B. The Obstetric Hematology Manual. New York: *Cambridge University Press*; 2010; 3-5.
12. Oliver E, Olufunto K. Management of Anemia in Pregnancy, Anemia, Silverberg D (Ed.) 2012. Available from: *http://www.intechopen.com/books/anemia/management-of-anaemia-in-pregnancy*. Accessed on January 29, 2015.
13. Cyril C, Hyacinth E. Prevalence of anemia among pregnant women in Enugu: Nigeria. *African J of blood res*. 2005, 33(7): 80-155.

14. Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in hematological parameters during pregnancy. *Indian J Hematol Blood Transfus*. 2012; 28(3): 144-146.
15. Vanderjagt DJ, Brock HS, Melah GS, El-Nafaty AU, Crossey MJ, Glew RH. Nutritional factors associated with anemia in pregnant women in northern Nigeria. *J Health Popul Nutr*. 2007; 25(1): 75-81.
16. World Health Organization. World wide prevalence of anemia1993-2005: WHO global database on anemia. Geneva: World Health Organization; 2008.
17. Verdy E, Bessous V, Dreyfus M, Kaplan C, Tchernia G, Uzan S. Longitudinal analysis of platelet count and volume in normal pregnancy. *Thromb Haemost*. 1997; 77: 806-807.
18. Michael J P, Hossain N. Hematological changes in pregnancy.US: *Black well*, 2010,1-4
19. Pughikumo OC, Pughikumo DT, Omunakwe HE. White Blood Cell Counts in Pregnant Women in Port Harcourt, Nigeria. *IOSR J Den Med Sci*. 2015; 14(3):1-3.
20. Verma A, Chaudhary H. Study of Hematological parameters in advanced pregnancy. *Inter J Recent Trends in Sci Tech*. 2013; 7(1): 16-19.
21. Sharma P, Nagar R. Hematological profile of anemic pregnant women attending antenatal hospital. *IOSR J Nursing and Health Sci*. 2013; 1(4): 11-15.
22. Kaur S, Khan S, Nigam A. Hematological profile and pregnancy: a review. *Int J Adv Med*. 2014; 1:68-70.
23. Dalal E, Shah J. A comparative study on outcome of neonates born to anemic mothers versus non anemic mothers. *Nat J Med Res*. 2014; 4(4): 270-273.
24. Nega D, Dana D, Tefera T, Eshetu T. Anemia associated with asymptomatic malaria among pregnant women in the rural surroundings of Arba Minch Town, South Ethiopia. *BMC Research Notes*. 2015; 8:110.
25. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014; 123(5):615-624.
26. Biswas M , Baruah R. Maternal anemia associated with socio-demographic factors among pregnant women of Boko-Bongaon Block Kamrup, Assam. *Indian J Basic Applied Med Res*. 2014: 3(2):712-721.

27. Haq HS, Asif M. Prevalence of anemia in pregnant women in Muzaffarabad division of Azad Jammu and Kashmir. *Ann Pak Inst Med Sci*. 2014; 10(1)39-42.
28. Ezzati M, Lopez AD, Rodgers AA, Murray CJL. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. Geneva, Switzerland: World Health Organization, 2004.
29. Charles CV, Summerlee AJS, Dewey CE. Anemia in Cambodia: prevalence, etiology and *research needs. Asia Pac J ClinNutr. 2012; 21 (2):171-181.*
30. Osungbade KO, Oladunjoye AO. Anemia in Developing Countries: Burden and Prospects of Prevention and Control. Anemia.2012; 8:116-125. Zehra T, Khan RA, Qadir F. Anemia in pregnancy a study of Karachi in a tertiary care centre. *American J PhytomedicineClin Therapeutics*. 2014; 2(10):1224-1233.
31. Ababiya T, Gabriel T. Prevalence of anemia among pregnant women in Ethiopia and its management: A review. *Int Res J Pharm*. 2014; 5(10):737-750.
32. WHO. Prevention and treatment of malaria during pregnancy. Available from: *http:/usaid.gov/pdf-docs/pnada621.pdf.2004*.[Accessed on Oct., 2015].
33. Richard F, Alexandre H. Thrombocytopenia in pregnancy. *Medcape J*. 2006. Available at *www.emedicine.medscape.com/article*. [Accessed 1 Feb 2016].
34. Osonuga IO, Osonuga OA, Onadeko AA, Osonuga A, Osonuga AA. Hematological profile of pregnant women in southwest of Nigeria. *Asian Pacific J Trop Dis*. 2011; 232-234.
35. Chaudhari SJ, Bodat RK. Tracking of hematological parameters in first and second trimester of pregnancy. *Nat J Med Res*. 2015; 5(3): 259-261.
36. Chaudhari SJ, Bodat RK. Are There any Difference in Hematological Parameters in Pregnant and Non – Pregnant Women?. *Ntl J of Community Med*. 2015; 6(3):429-432.
37. Okpokam DC, Okhormhe ZA, Ernest NA, Udoh KN, Akpotuzor JO, Emeribe AO. Comparative study of some hematological parameters of pregnant women in Akpabuyolocal government area of Cross River State, Nigeria. *Der Pharmacia Lettre*. 2015; 7 (7):1-5.
38. Ichipi-Ifukor PC, Jacobs J, Ichipi-Ifukor RN, Ewrhel OL. Changes in hematological indices in normal pregnancy. *Physiology Journal*. 2013; 1-4.

39. Ifeanyi OE, Ndubuisi OT, Leticia EOB, Uche EC. Hematological profile of pregnant women in Umuahia, Abia State, Nigeria. *Int.J.Curr.Microbiol.App.Sci*. 2014; 3(1): 713-718.
40. Purohit G, Shah T, Harsoda JM. Hematological profile of normal pregnant women in Western India. *Sch.J.App.Med.Sci*. 2015; 3(6): 2195-2199.
41. Das S, Char D, Sarkar S, Saha TK, Biswa S. Study of Hematological Parameters in Pregnancy. *IOSR J Den Med Sci*. 2013; 12(1):42-44.
42. Belayneh F, G/Mariam A, Solomon F, Geto Z, Amsalu A, Nigussie D, et al. Prevalence of Thrombocytopenia, and Associated factors among pregnant women attending antenatal care at Hawassa University Teaching and Referral Hospital. *J Harmoniz Res Med Hlth Sci*. 2015; 2(4): 175-182.
43. WHO. The global prevalence of anemia in 2011. Geneva: World Health Organization; 2015.
44. Ethiopia Demographic and Health Survey. Addis Ababa, Ethiopia: Central Statistics Agency; 2011.
45. Jufar AH, Zewde T. Prevalence of Anemia among Pregnant Women Attending Antenatal Care at Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia. *J Hematol Thromb Dis*. 2014; 2: 1-6.
46. Ejeta E, Alemnew B, Fikadu A, Fikadu M, Tesfaye L, Birhanu T. Prevalence of anemia in pregnant women's and associated risk factors in Western Ethiopia. *Food Science and Quality Management*. 2014; 31:82-91.
47. Kefiyalew F, Zemene E, Asres Y, GedefawL. Anemia among pregnant women in Southeast Ethiopia: prevalence, severity and associated risk factors. *BMC Research Notes*. 2014; 7:771.
48. Abriha A, Yesuf ME, Wassie MM. Prevalence and associated factors of anemia among pregnant women of Mekelle town: a cross sectional study. *BMC Research Notes*. 2014; 7:888.
49. Gedefaw L, Ayele A, Asres Y, Mossie A. Anemia and associated factors among pregnant women attending antenatal care clinic in Wolayita Sodo Town, Southern Ethiopia. *Ethiop J Health Sci*. 2015; 25(2): 155-162.

50. Hailemariam S, Tenkolu G, Ayele HT. Malnutrition: Prevalence and its associated factors in people living with HIV/AIDS, in Dilla University Referral Hospital. Activities of public health. 2013; 71:13
51. Elgari MM. Evaluation of Hematological Parameters of Sudanese Pregnant Women attending at Omdurman Al Saudi Maternity Hospital. Egypt. *Acad. J. Biolog. Sci*. 2013; 5(1):37-42.
52. James TR, Reid HL, Mullings AM. Are published standards for hematological indices in pregnancy applicable across populations: an evaluation in healthy pregnant Jamaican women? *BMC Pregnancy and Childbirth*. 2008; 8 (8):1-4.
53. Pitkin RM, Witte DL. Platelet and leucocyte count in pregnancy. *J Pakistan med assoc*. 1979; 242 (24): 2696-2698.
54. Luppi P. How immune mechanisms are affected by pregnancy. *Vaccine*. 2003; 21 (24): 3352-3357.
55. Melku M, Addis Z, Alem M, Enawgaw B. Prevalence and Predictors of Maternal Anemia during Pregnancy in Gondar, Northwest Ethiopia: An Institutional Based Cross-Sectional Study. *Anemia*. 2014; Article ID 108593.
56. Gies S, Brabin BJ, Yassin MA, Cuevas LE. Comparison of screening methods for anaemia in pregnant women in Hawassa, Ethiopia. *Trop Med Int Health*.2003; 8(4):301–309.
57. Alem M, Enawgaw B, Gelaw A, Kena T, Seid M, Olkeba Y. Prevalence of anemia and associated risk factors among pregnant women attending antenatal care in Azezo Health Center Gondar town, Northwest Ethiopia. *J Inter disciple Histopathol*. 2013; 1(3): 137-144.
58. Gebre A, Mulugeta A. Prevalence of anemia and associated factors among pregnant women in north western zone of Tigray, Northern Ethiopia: A Cross-Sectional Study. *J Nutr Metabol*.2015, Article ID 165430.
59. Obse N, Mossie A, Gobena T. Magnitude of anemia and associated risk factors among pregnant women attending antenatal care in shalla woreda, West Arsi Zone, Oromia Region, Ethiopia. *Ethiop J Health Sci*. 2013; 23(2):165-173.

60. Bekele A, Tilahun M, Mekuria A. Prevalence of Anemia and It's associated factors among pregnant women attending antenatal care in health institutions of Arba Minch Town, Gamo Gofa Zone, Ethiopia: A Cross-sectional study. *Anemia*. 2016; 1-9.
61. Getachew M, Yewhalaw D, Tafess K, Getachew Y, Zeynudin A. Anemia and associated risk factors among pregnant women in Gilgel Gibe dam area, Southwest Ethiopia. *Parasites & Vectors*. 2012; 5:296.
62. Lelissa D, Yilma M, Shewalem W, Abraha A, Worku M, Ambachew H,et.al.: Prevalence of Anemia Among Women Receiving Antenatal Care at Boditii Health Center, Southern Ethiopia. *Clin Med Res*. 2015; 4(3): 79-86.
63. Mihiretie H, Fufa M, Mitiku A, Bacha C, Getahun D, Kejela M, et al. Magnitude of Anemia and Associated Factors among Pregnant Women Attending Antenatal Care in Nekemte Health Center, Nekemte, Ethiopia. *J Med Microb Diagn*.2015; 4: 197.
64. Karaoglu L, Pehlivan E, Egri M, Deprem C, Gunes G, Genc MF. The prevalence of nutritional anemia in pregnancy in an east Anatolian province, Turkey. *BMC Public Health.* 2010; 10:329.
65. Manjulatha B, Padmasri P, Sravanthi TP. Prevalence of Anemia in Pregnant Women in Tertiary Care Centre. *IOSR J Den Med Sci*. 2015; 14 (I 7): 91-95.
66. Van den Broek N. Anemia and micronutrient deficiencies. *Br Med Bull*. 2003; 67: 149-160.
67. Woldemariam G, Timotiows G. Determinants of Nutritional Status of Women and Children in Ethiopia, Calverton, Maryland, USA: 2002; 49-57.

11. ANNEX

ANNEX I. Participant Information Sheet

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences

Title of the study: Hematological Profile of Pregnant Women Attending Antenatal Care at Dilla University Referral Hospital, Dilla, Ethiopia: Comparative Cross Sectional Study

Introduction

This information sheet and consent form is prepared by the principal investigator to clarify the study that you are asked to take part. If there is any lack of clarity before you decide to participate or not you can ask freely.

What are expected from participant?

You will be requested to give 3ml (spoon full) of blood and 1gm (pea) of stool. Blood will be collected from your arm using sterile syringe. If you are agree to give sample you will be also requested to answer for questionnaire

Confidentiality

Any information that we collect about you during this research will be kept confidential. Information about your identity will be put away after recording your file; and kept in a secured place. Only the principal investigators will be able to link your identity with the code number.

Benefit

Based on the diagnosis result you will be treated accordingly. On the other hand, the result of the study will be beneficial to put a new strategy for control of pregnancy complications. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Right to refuse

We are asking you and others to voluntarily participate in this study. Since participation in this study is entirely voluntary. You can refuse to participate in this research at any time. Your

refusal to participate in this study will not affect any of the benefits you are supposed to get from the center.

Please direct any questions or problems you may encounter during this study to the principal investigator:

Sara Anberbir

Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Phone: +251-0911097548

Email: anberbir62@gmail.com

Amharic version Participant information sheet

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ት/ክፍል

ለጥናቱ ተሳታፊ መግለጫ

ርእስ፡ በዲላ ዩኒቨርሲቲ ሪፈራል ሆስፒታል የነፍሰጡሮች ሄማቶሎጂካል ፕሮፋይል

መግቢያ

- ይህ መግለጫና የጥናቱ ማረጋገጫ ቅጽ የተዘጋጀዉ በዋናዉ ተመራማሪ ሲሆን በጥናቱ ላይ ለመሳተፍ ከመወሰኖ በፊት ግልጽ ያልሆነልዎትን መጠየቅ ይችላሉ፡፡

ከጥናቱ ተሳታፊ ምን ይጠበቃል?

- የጥናቱ ተሳታፊ እንደመሆንዎ አንድ ማንኪያ የሚሆን የደም ናሙና እና አተር ፍሬ የሚያክል አይነምድር እንዲሰጡ ይጠየቃሉ። ደሙ ከክንድዎ በአዲስ መርፌ እና ስሪንጅ የሚቀዳ ይሆናል። ናሙናውን መስጠት ፍቃደኛ ከሆኑ ለሚቀርብልዎ ጥያቄዎች መልስ እንዲሰጡ እንደገና ይጠየቃሉ።

የጥናቱ መረጃ ሚስጥራዊነት

- በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም ግላዊ መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡ ከማንነትዎ ጋር በቀጥታ ተያያዝነት ያላቸው መረጃዎች በሙሉ በዋናው ተመራማሪ ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚውሉ ይሆናሉ፡፡

ጠቀሜታ

- በናሙናው ዉጤት መሰረት ይታከማሉ። በተጨማሪም የጥናቱ ዉጤት ከእርግዝና ጋር ተያይዞ የሚመጡ ችግሮችን ለመቆጣጠር የራሱ የሆነ ጥቅም ይኖረዋል።ስለሆነም የጥናቱ ተሳታፊዎች በተዘዋዋሪ መንገድ ለሌሎች ነፍሰጡሮች ብሎም ለህብረተሰቡ ይጠቅማሉ፡፡

በጥናቱ ላይ የመሳተፍ እና ያለመሳተፍ

- በዚህ ጥናት ላይ መሳተፍ በሙሉ ፍቃደኝነት ላይ የተመሰረተ ነው ስለሆነም በጥናቱ እንዲሳተፉ ፍቃደኝነትዎን እንጠይቃለን። ይህ ጥናት በፍቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛውም ወቅት በፍቃድዎ ከጥናቱ መውጣት ይችላሉ፡፡ ከጥናቱ ራስዎን ቢያገሉም እንኳን ከተቋሙ ማግኘት ያለቦት እርዳታ ምንም አይጓደልቦትም፡፡
- ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ ካልዎት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ሳራ አንበርብር

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል: +251-0911097548

ኢ-ሜይል፡ anberbir62@gmail.com

ANNEX II. Consent Form

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I give consent to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand.

Participant ID---------- Signature: ----------Date ----------

Name of person obtaining consent---------- Signature ----------Date ----------

Amharic version consent form

እኔ ስሜ ከታች የፈረምኩት የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱ አላማዎች አሰራሮች እና ቅድመ ሁኔታዎች በግልጽ በመረዳት እና ለጥናቱ ተሳታፊነት ፍቃደኝነቴን በማንኛውም ደረጃ የማንሳት መብቴን በማረጋገጥ ነው፡፡ በመሆኑም በጥናቱ ተሳታፊ ለመሆን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎች በሚገባ የተረዳሁና ከጥናቱ በማንኛውም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ እገዛዎች ሁሉ እንደማይነፈጉኝ በማመን ነው፡፡ እነዚህን መረጃዎች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው መለያ ቁጥር---------- ፊርማ----------ቀን----------

ስምምነቱን የተቀበለው ሰው ስም ----------ፊርማ---------- ቀን----------

ANNEX III. Questionnaire

Addis Ababa University health Science College

Department of Medical Laboratory Science

For data collectors: For each question please circle the answer don't worry if you make a mistake; simply cross out a mistake and circle the correct one.

ANC No…… STUDY No...……..

Questionnaire on Socio demographic, dietary habit and other relevant characteristics of the respondent

1. Age ----------
2. Residence A. Urban B. Rural
3. What is your educational status?

   A. Illiterate B. Primary school

   C. Secondary school D. College/university

4. What is your occupation?

   A. Private B. government

   C. house wife D. other specify ----------

5. How much is your monthly income (in average) in birr?

   A. Below 500 B. 501 -850

   C. 851 -1200 D. Above 1201

6. Frequency of eating animal food products.

   A. I don't eat B. daily

   C. Every other day D. Once per week E. once in month

7. Frequency of eating green leaf vegetables.

A. I don't eat B. daily

C. Every other day D. Once per week E. once in month

8. Taking tea/ coffee immediately after meal

A. Yes B. No

9. Frequency of Taking fruits

A. I don't eat B. daily

C. Every other day D. Once per week E. once in month

10. How much is your gestational age in months/weeks? ----------

Amharic version questionnaire

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ

የህክምና ላቦርቶሪ ት/ ክፍል

ለመረጃ ሰብሳቢዎች፤ ጥያቄውን ከጠየቃችሁ በኃላ መልሱን ከተሰጡት አማራጮች አንዱን ያክቡ፤ ምን አልባት ስህተት ቢፈጠር በመሰረዝ በምትኩ ትክክለኛውን ምላሽ ያክቡ፡፡

መለያ ቁጥር---------- የጥናት ቁጥር----------

ማህበራዊ፤ ኢኮኖሚያዊና ሌሎች ተያያዝ የሆኑ መጠይቆች

1. እድሜዎ ስንት ነዉ?
2. የሚኖሩበት አካባቢ የት ነዉ?

   ሀ. ከተማ ለ. ገጠር
3. የትምህርት ደረጃዎት ስንት ነዉ?

   ሀ. ምንም ያልተማረ ለ. አንደኛ ደረጃ

   ሐ. ሁለተኛ ደረጃ መ. ኮሌጅ/ ዩኒቨርሲቲ
4. ስራዎት ምንድነዉ?

   ሀ. የግል ለ. የመንግስት

   ሐ. የቤት እመቤት መ. ሌላ ይጠቀስ
5. የወር ገቢዎ በአማካኝ ስንት ይሆናል?

   ሀ. ከ500 በታች ለ. 501 - 850

   ሐ. 851 -1200 መ. ከ 1201 በላይ
6. የእንስሳት ተዋጽዎ የሆኑ ምግቦችን በምን ያህል ጊዜ ይመገባሉ?

   ሀ. አልመገብም ለ. በየቀኑ ሐ. በየሁለት ቀን

   መ. በሳምንት አንዴ ሠ. በወር አንዴ
7. አትክልት ምግቦችን በምን ያህል ጊዜ ይመገባሉ?

   ሀ. አልመገብም ለ. በየቀኑ ሐ. በየሁለት ቀን

   መ. በሳምንት አንዴ ሠ. በወር አንዴ
8. ሻይ/ ቡና ከምግብ በኋላ ይወስዳሉ?

   ሀ. አዎ ለ. አልወስድም
9. ፍራፍሬ ምግቦችን በምን ያህል ጊዜ ይመገባሉ?

ሀ. አልመገብም ለ. በየቀኑ ሐ. በየሁለት ቀን

መ. በሳምንት አንዴ ሠ. በወር አንዴ

10. እርግዝናዎ ምን ያህል ወር/ሳምንት ይሆነዋል?

ANNEX IV. Venous Blood Collection

Supplies and Equipment

1.Tourniquet and disposable gloves
2.Alcohol (70%) and gauze square or alcohol wipes
3.Sterile disposable needles (double-pointed or syringe type)
4. Evacuated blood tubes (appropriate to the test ordered) and a needle holder or a syringe (in special cases)

Procedure

1. Identify the patient
2. Assemble all necessary equipment
3. Visually inspect both arms. Choose the arm that has not been repeatedly used for vein punctures and one that is free of bruises, abrasions, and sites of infection. In the arm, three veins are commonly used for vein puncture: the cephalic, basilic, and median cubital.
4. Applying the tourniquet
5. Using a cotton ball saturated with 70% alcohol or an alcohol pad saturated with 70% alcohol, cleanse the skin in the area of the vein puncture site. Using a circular motion, clean the area from the center and move outward. Do not go back over an area once it has been cleansed. Allow the site to dry.
6. Use one hand to hold the evacuated tube assembly or syringe. Use one or more fingers of the other hand to secure the skin area of the forearm below the intended vein puncture site. This will tighten the skin and secure the vein. Position the patient arm in a slightly downward position.
7. Hold the needle with attached syringe or evacuated tube about 1 to 2 inches below and in a straight line with the intended vein puncture site. Position the blood drawing unit at an angle of about 20°. The bevel of the needle should be upward.
8. Gently insert the needle through the skin and into the vein. The insertion motion should be smooth.

9. The tourniquet may be released as soon as the blood begins to flow into the evacuated tube or syringe or immediately before the final amount of blood is drawn. Ask the patient to open the hand.
10. After the desired amount of blood has been drawn, place a gauze pad over the vein puncture site.
11. Withdraw the blood collecting unit with one hand and immediately press down on the gauze pad with the other hand.
12. If possible, have the patient elevate the entire arm and press on the gauze pad with the opposite hand. If the patient is unable to do this, apply pressure until bleeding ceases.
13. Place a non-allergenic adhesive spot or strip over the vein puncture site. Note: Failure to apply sufficient pressure to the vein puncture site could result in a hematoma (a collection of blood under the skin that produces a bruise).
14. Mix tubes with anticoagulant by inverting the tubes several times. If a syringe was used, carefully remove the needle before dispensing the blood into a test tube. Blood should never be forced back through the needle, and the syringe plunger should be slowly depressed. Discard the used needle into an appropriate safety container.
15. Label all test tubes as required by the laboratory.
16. Clean up supplies from the work area, remove gloves, and wash hands. Note: If the patient is an outpatient, wait a few minutes after the vein puncture is complete, and check to be sure that the patient does not feel dizzy or nauseated before discharge. Discard all contaminated supplies in a biohazard disposal bag.

ANNEX V. Cell-Dyn 1800 Hematology Analyzer

Specimen Requirements

1. Whole blood collected in an EDTA tube.
2. Minimum sample volume is 0.5 mL using the Open Sample Mode. The instrument aspirates 30 µL of patient sample.
3. Samples are stable at room temperature for eight hours.

Cause for rejection

- Hemolysis
- clotted specimen
- Tube not filled with minimum volume
- Improperly labeled specimen.

Reagent

- ❖ Cyanide-Free Diff Lyse Reagent,
- ❖ Detergent,
- ❖ Diluents and
- ❖ Enzymatic Cleaner.

Reagent stored at room temperature except Enzymatic Cleaner which should be stored between 2 and 8 degree centigrade.

Procedure to perform patient testing

1. 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.
2. Enter specimen number and patient name in the MRN and Patient Name using the keyboard.
3. With the cap tightly secured on the specimen tube, slowly invert the tube 8 times.
4. Remove the cap from the pre-mixed specimen tube.

5. 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.
7. 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. A beep will indicate that the probe cleaning cycle has begun.
8. 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.
9. If needed, press PRINT REPORT for a hardcopy of the report.
10. After sampling is complete, press MAIN to return to the MAIN MENU. Change the Operator ID to “000”for the next user.

Quality control

Quality control checks performed daily according to the laboratory’s protocol. Commercial controls materials are properly warmed and mixed according to the manufacturers’ recommendations. Patient controls are handled according to the laboratory’s protocol.

ANNEX VI. Blood smear preparation and staining

Technique of making thin blood film

1. Label a clean dry microscope slide
2. Place a small drop of well mixed EDTA 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
   - ✓ when the blood is from an anemic patient larger drop of blood should be used
3. Using a clean smooth edged spreader, draw the spreader back to touch the drop of blood and allow the blood to extend along the edge of the spreader. Holding the spreader at an angle of about 45^0, spread the drop of blood to make a film about 40-50 mm in length (two thirds of the slide). Wipe clean the end of the spreader.
4. Immediately air dried the film. Protect the dried film from dust and insects. Do not blow on the smears as this can disrupt cellular morphology and cause the formation of unwanted artifacts, like target cells. Also do not use heat for drying

   Note: When not using a frosted ended slide, write the patient's name and number on the dried blood at the top or along the side of the film using a lead pencil.
5. When completely dry and within a few minutes of making the blood film, fix a thin film in absolute methanol.

Technique of making thick blood film

1. Place a small drop of blood in the center of the pre-cleaned, labeled slide.
2. Using the corner of another slide or an applicator stick spread the drop in a circular pattern until it is the size of a dime.
3. Lay the slides flat and allow the smears to dry thoroughly, protect from dust and insects.
4. Air dry at room temperature, need minimum of 30 minutes. Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to hemolyse the RBCs.

Geimsa staining procedure

Material

- ✓ Buffered water(pH7.1-7.2) or Buffered saline water, pH 7.1-7.2
- ✓ Geimsa staining solution

1. Place the air-dried smear film side up on a staining rack (two parallel glass rods kept 5cm apart).
2. Cover the air dried smear with a 1: 10 diluted Geimsa using buffered distilled water at pH 7.2 (recommended for malaria parasites in order to stain schuffners granules) as a diluent
3. Allow the diluted stain to act for 10 minutes
4. Wash off the stain with running tap water/wash bottle. Don't tip off the stain, because this will leave a fine deposit covering the film.
5. Wipe the back of the slide clean and stand it in a draining rack for the smear to dry (head part down).
6. Dry with air and do microscopy
7. The blood films are initially viewed with 10x objective, to find the best area where RBCs are evenly distributed, just touching but not overlapping. Then use the 100x (oil immersion) objective.

QUALITY CONTROL

- ➢ To ensure that proper staining results have been achieved, stain positive smear (malaria) with each new batch of working Geimsa stain.

Annex VII. Stool sample collection and examination

Material

- ✓ 0.85 % normal saline
- ✓ Stool cup
- ✓ Applicator stick
- ✓ microscope
- ✓ slide,cover slide

Specimen collection

Collect the stool in a dry, clean, leak proof container. Make sure no urine, water, soil or other material gets in the container.

The specimens should be collected before administrations of anti acids, kaolin, mineral oil, non-absorbable anti diarrheal preparations, barium or bismuth (7-10 days needed for clearance of effects), antimicrobial agents (2-3 weeks), since these substances render the stool specimen unsatisfactory for examinations

Fresh stool should be examined, processed, or preserved immediately

Examination procedure

1. Obtain a microscope slide and the stool specimen.
2. Take a small amount of the specimen and place it on a microscope slide.
3. If the stool specimen is solid, add a drop or two of saline to the specimen and mix. Ideally, two smears can be prepared on one slide, of which one can be stained with iodine.
4. cover with cover slip to keep the organism form moving and prevent the preparation from drying out
5. Scan the entire cover slip area using the 10× objective, if something suspicious is seen, a higher magnification may be necessary.

Annex VIII: Declaration

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

Name Sara Anberbir (BSc)

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission ____/ ____/ ____

This thesis has been submitted with my approval as University Advisor

Name of advisor Mintwab Hussein (MSc, BSc)

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Name of advisor Melatwork Tibebu (MSc, BSc)

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia