ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Determinants and Effects of Iron Folate Supplementation on Maternal Hematological Indices among Pregnant Women in Addis Ababa, Ethiopia

By: Zeleke Endalew Admas, MSc

Advisors:

Diresibachew Haile, PhD

Abebaye Aragaw, PhD

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc) in Medical Physiology.

June 2024

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

School of Medicine

Department of Medical Physiology

Determinants and Effects of Iron Folate Supplementation on Maternal Hematological Indices among Pregnant Women in Addis Ababa, Ethiopia

Principal Investigator (PI): Zeleke Endalew Admass, MSc

Advisors: Diresibachew Haile, PhD

Abebaye Aragaw, PhD

Address of the PI: Cell Phone: +251-910-804-000

Email: zeleke.endalew@aau.edu.et

APPROVAL SHEET

This thesis by Zeleke Endalew Admas, is accepted in its present form by the board of examiners, satisfying the thesis requirement for the degree of Master of Science in Medical Physiology.

Internal Examiner

Name	Signature	Date
Muluken Walle, PhD	----------	----------

External Examiner

Name	Signature	Date
Mihret Getnet	----------	----------

Research Advisors

Name	Signature	Date
Diresibachew Haile, PhD.	----------	----------
Abebaye Aragaw, Ph.D	----------	----------

Department Head

Name	Signature	Date
Abebaye Aragaw, Ph.D	----------	----------

DECLARATION

I, the undersigned postgraduate student, declare that this thesis work entitled "Determinants and Effects of Iron Folate Supplementation on Maternal Hematological Indices among Pregnant Women in Addis Ababa, Ethiopia" is my work and that all the resources that I have used or quoted have been cited and acknowledged accordingly. This work has not been undertaken by anyone nor submitted to any other institution.

Principle Investigator

Name	Signature	Date
Zeleke Endalew Admas		22/07/2024

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who have contributed to the successful completion of my master's thesis writing. Without their support, this accomplishment would not have been possible.

Firstly, I would like to thank my supervisors Diresibachew Haile, PhD and Abebaye Aragaw, PhD for their invaluable guidance, encouragement, and feedback throughout the thesis writing process. Their knowledge, expertise, and insight have been critical to the development and refinement of my research writing. Moreover, I am grateful to Addis Ababa University for sponsoring this research work.

My appreciation also goes to Dilla University for allowing me to pursue a postgraduate degree in Medical Physiology. Moreover, I am grateful to the faculty and staff of Addis Ababa University Physiology Department, whose expertise, resources, and support have been instrumental in the development of my academic and research skills. I also extend my sincere gratitude to the data collectors and participants of this study for their time and valuable contributions.

Finally, I am grateful to my family and friends for their unwavering support and encouragement throughout my academic journey. Their love, patience, and understanding have been a constant source of motivation and inspiration.

Thank you all for your contributions, encouragement, and support.

ABBREVIATIONS/ACRONYMS

ANC	Ante Natal Care
AOR	Adjusted Odds Ratio
CSA	Central Statistical Agency
CI	Confidence Interval
EDHS	Ethiopia Demography and Health Survey
EPHI	Ethiopian Public Health Institute
FL	Femto Liter
FPN	Ferroportin
FMOH-E	Federal Ministry of Health, Ethiopia
GA	Gestational Age
Hb	Hemoglobin
HIFs	Hypoxia-Inducible Factors
HTN	Hypertension
IDA	Iron Deficiency Anemia
IFAS	Iron-Folic Acid Supplementation
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MCHC	Mean Cell Hemoglobin Concentration
MDD-W	Minimum Dietary Diversity for Women
RBCs	Red Blood Cells
RCTs	Randomized Controlled Trials
RDW	Red Cell Distribution Width
RECs	Reticulo-Endothelial cells
ROS	Reactive Oxygen Species
STfR	Soluble Transferrin Receptor
Th2	T Helper Cell Type 2
TIBC	Total Iron Binding Capacity

LIST OF TABLES

LIST OF FIGURES

CONTENTS

ABSTRACT

Background: Iron-folate (IFA) supplementation is a well-established strategy for reducing anemia in pregnant women. Studies on the extent to which it reduces maternal anemia, its effect on leukocytes and platelet count are limited and require further investigation.

Objective: This study aimed to assess determinants and effects of iron folate supplementation on maternal hematological indices among pregnant women in public hospitals in Addis Ababa, Ethiopia.

Methods: An institution-based prospective cohort study was conducted in public hospitals in Addis Ababa from May 1, 2023, to March 30, 2024, with 410 participants selected through systematic random sampling. Data collection involved interviews, medical record reviews, laboratory tests, anthropometry, and blood pressure measurements. Statistical analysis was performed using SPSS Version 27, including Shapiro-Wilk test, descriptive statistics, paired sample t-tests, and Wilcoxon signed-rank tests. A p-value of less than 0.05 was considered significant. Logistic regression and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were used to identify significant associations.

Results: RBC Count (in 10^6 cells/µl) changed from the baseline value of 4.51±0.43 to 4.70±0.54 (P<0.001) in non-anemic respondents while it increased from 4.05±0.37 to 4.23±0.39 (P<0.001) in anemic respondents. Also, platelet count (in 10^3 /µl) decreased from 293.3±37.01 to 285.4±37.83 (P<0.01) in anemic respondents and from 274.9±56.3 to 254.29±59.07 in non-anemic pregnant women (p<0.001). After supplementation, no significant change in leukocyte count was observed in both anemic and non-anemic pregnant women (p=0.065 and p<0.059) respectively. Inadequate hemoglobin response to iron folate supplementation was found in 59.7 % of the respondents. Early ANC booking (AOR= 3.9, 95% CI: 2.4 - 4.2), IFA intake for more than 2 months (AOR = 2.6, 95% CI: 1.6 - 4.2), adequate dietary diversity (OR =3.4, 95% CI: 2.1 - 5.6), and primiparity (OR = 2.4, 95% CI: 1.4 - 4.2) were significantly associated with adequate hemoglobin response to iron folate supplementation.

Conclusion: Iron-folate supplementation increased red blood cell count in pregnant women, but its impact on platelet count requires further investigation. This study underscores the importance of considering dietary diversity, parity, early prenatal care, and adherence to achieve adequate hemoglobin response to iron folate supplementation.

Key words: Anemia, blood cell indices, iron deficiency, iron-folate supplementation, pregnancy

1. INTRODUCTION

1.1 Background

Pregnancy involves changes in maternal physiology, including alterations in hematologic parameters, that occur to meet the demands of the developing fetus and placenta [1]. During pregnancy, requirements for micronutrients including iron and folate, increase to maintain maternal homeostasis while supporting fetal growth. The increased demand for these micronutrients coupled with the increase in plasma volume to red blood cell mass ratio predisposes the mother to anemia [2].

Anemia is a condition in which the number of red blood cells or the blood hemoglobin concentration is lower than normal, resulting in a decreased capacity of the blood to carry oxygen to the body's tissues [3]. It is a serious global public health problem affecting pregnant women particularly [4,5]. It manifests with symptoms including but not limited to fatigue, weakness, dizziness and shortness of breath. The physiological Hb concentration needed to satisfy the physiologic needs varies by age, sex, elevation of residence, smoking habits and pregnancy status [6]. The most common causes of anemia include nutritional deficiencies, particularly iron deficiency. Deficiencies in folate, vitamins B12 and A; Haemoglobinopathies, infectious diseases like malaria, tuberculosis, HIV and parasitic infections can also cause anemias [7–9].

According to the WHO guideline, anemia in pregnancy is defined as Hb levels < 11.0 g/dl in the first and third trimester of pregnancy and < 10.5 g/ dl in the second trimester of pregnancy [10]. However, cut off value of 11.0 g/dl has been used for anemia independent of gestation in most circumstances [9]. Using red cell size as a criterion, anemia can be classified as microcytic, normocytic, and macrocytic anemia [3,9]. Microcytic anemias occur when the mean corpuscular volume (MCV) is less than normal (<80 (fL)). This occurs in conditions of iron deficiency or malabsorption of Vitamin B12/iron, and genetic defects in hemoglobin synthesis (thalassemia, sickle cell disease) [3,9]. Macrocytic anemia (MCV >100 fL) is mainly caused by vitamin B12 and folate deficiency, and liver diseases [9]. Normocytic anemia (MCV 80–100 fL), on the other hand, is mainly due to chronic infection, inflammation and hemolysis or blood loss [11]. Based on the underlying mechanism anemia can also be classified as hypo proliferative (i.e. decrease in RBC production) or maturational abnormalities (i.e. increase in RBC loss or destruction) [12,13].

Iron deficiency being the most common micronutrient deficiency in the world, is the cause for half of the anemia reported in pregnant women and disproportionately affects pregnant women [14,15]. In Ethiopia, daily iron supplementation for at least 6 months (depending on the severity of the condition) during pregnancy and 3 months postpartum, iron supplementation is an integral part of Antenatal Care (ANC) and postnatal care services [16]. Iron-folate supplementation has a plethora of benefits to the mother and the fetus such as reducing the risk of maternal anemia, maternal mortality, the risks of premature birth and low birth weight [17].

1.2 Statement of the problem

Iron and folate deficiency during pregnancy are critical public health concerns that can lead to severe maternal and fetal health outcomes. Despite the availability of iron-folate supplementation, anemia remains prevalent among pregnant women in many parts of the world, including Ethiopia. Anemia during pregnancy is primarily caused by iron deficiency, which can result in adverse outcomes such as preterm birth, low birth weight, and increased maternal mortality [18]. In iron replete pregnant women, excess iron resulting from iron supplementation has also been reported to cause adverse health outcomes [19]. Folate deficiency, on the other hand, is associated with anemia, neural tube defects and other congenital abnormalities [18]. Despite the critical importance of these nutrients, studies indicate that a significant number of pregnant women do not receive adequate supplementation, resulting in suboptimal hematological indices [20].

Anemia is estimated to affect 36.8 % of pregnant women globally [21]. The highest prevalence of anemia in pregnant women reported is in Africa with a prevalence of 41.7% [21]. In sub-Sahara African countries, it is reported to affect 44.3% of pregnant women. In Ethiopia, the prevalence of anemia among pregnant women is alarmingly high. It has also been reported that approximately 41% of pregnant women in Ethiopia are anemic, with iron deficiency being the most common cause [22]. This high prevalence is concerning given the well-documented benefits of iron-folate supplementation in improving maternal health outcomes [23].

The determinants of iron-folate supplementation uptake and effects are multifaceted, involving socio-economic, dietary, altitude-related, and health system-related factors [24]. Socio-economic factors such as low income and educational status significantly impact the ability of pregnant women to access and adhere to iron-folate supplementation [25]. Women from lower socio-economic backgrounds may face barriers in affording or accessing supplements, while those with higher educational attainment may have better knowledge about the importance of supplementation. Cultural beliefs and practices also play a role, with some women avoiding supplementation due to misconceptions about side effects

or mistrust in medical advice [26]. These cultural barriers can significantly hinder the effectiveness of public health interventions aimed at improving iron and folate intake during pregnancy.

Dietary habits are another crucial factor affecting hematological indices. Inadequate dietary intake of iron and folate can exacerbate deficiencies, especially in populations where the diet is predominantly plant-based and low in bioavailable iron [27]. Diets may lack sufficient heme iron sources, which are more readily absorbed by the body compared to non-heme iron found in plant foods.

Addis Ababa's high altitude (approximately 2,355 meters above sea level) can also influence the hematological outcomes. High-altitude environments are associated with increased hemoglobin concentrations due to hypoxia-induced erythropoiesis [28,29]. However, this adaptive mechanism can sometimes mask underlying anemia and complicate the assessment of iron status [30,31]. The increased physiological demand for oxygen in pregnant women at high altitudes may exacerbate iron and folate deficiencies if not adequately addressed.

Health system-related barriers such as inadequate supply of supplements, poor quality of antenatal care, and lack of health education contribute to low supplementation rates [32]. Effective health education programs that emphasize the importance of iron-folate supplementation and address misconceptions are essential for improving adherence rates. The effectiveness of iron-folate supplementation is further influenced by the timing and dosage of the intervention. Studies suggest that supplementation initiated early in pregnancy and continued throughout the gestation period is more effective in improving hematological indices [33].

Despite the universal iron folate- supplementation during pregnancy that is being implemented, the prevalence of anemia and hence iron deficiency is high. To address these challenges, it is imperative to understand the specific determinant factors that affect the effect of iron-folate supplementation on maternal hematological status among pregnant women. There is a critical need for comprehensive data on the hematological impact of iron-folate supplementation among iron replete pregnant women. Understanding the determinants and effects of iron-folate supplementation on maternal hematological indices is crucial for developing effective interventions. Moreover, studies on the effect of iron folate supplementation on maternal hematological indices is limited. Furthermore, Factors like altitude and dietary habits have not been explored much. This gap in knowledge underscores the need for rigorous research to inform policy and practice. This research aims to fill the existing gaps on IFAS and associated factors on hematological parameters.

1.3 Significance of the study

Data on the effect of IFA supplementation on maternal hematological indices and other relevant determinant factors in Addis Ababa in pregnant women is limited. This could limit the development and effectiveness of anemia prevention efforts in the country. In the study area, factors that could contribute to iron deficiencies, such as altitude and dietary diversity, have not been studied much yet. Moreover, the available few researches on the topic showed that there is a high prevalence of anemia in pregnant women despite the universal IFA supplementation strategy employed by the Federal Ministry of Health-Ethiopia. Therefore, this study is designed to pinpoint the factors associated with the reported high post-supplementation prevalence of anemia.

The findings of this study could serve as a baseline or source of information for other researchers interested in this area. It could also inform authorities and program policy/decision makers on how to improve IFAS coverage and adherence among pregnant women, contribute to the global knowledge on the effectiveness of IFAS in improving maternal hematological status, provide evidence on the impact of IFAS on maternal hematological status and identify barriers and facilitators to IFAS compliance in Ethiopia.

2. LITERATURE REVIEW

1.1 Micronutrient requirements during pregnancy

Micronutrients are essential vitamins and minerals that are required in small quantities to support metabolic activities including cell signaling, motility, proliferation, differentiation and apoptosis that regulate tissue growth, function and homeostasis [34]. These essential micronutrients include iron, zinc, vitamins C, vitamin E and B-complex vitamins including folic acid (i.e. folate) [35]. Adequacy of these micronutrients is critical especially during the periconceptional period, throughout pregnancy and lactation [36].

Iron is crucial for the function of all cells, playing a role in oxygen delivery, electron transport, and enzymatic activity [3]. It is not synthesized within the body and therefore must be absorbed from dietary sources [37]. In the human body, iron exists mainly in erythrocytes as a heme compound (i.e. hemoglobin), in storage compounds (i.e. ferritin and hemosiderin) and in muscle cells as myoglobin [9]. Iron is also found bound to proteins (i.e. hemoprotein) and non-heme enzymes involved in oxidation-reduction reactions and the transfer of electrons (cytochromes and catalase) [38]. Dietary iron exists in heme and non-heme forms. Heme iron, derived from hemoglobin and myoglobin of animal food sources (i.e., meat, seafood, poultry), is the most easily absorbable form (15% to 35%) and contributes 10% or more of our total absorbed iron. The non-heme iron, on the other hand, is derived from plants and iron-fortified foods and is less well absorbed [38].

The absorption of dietary iron occurs in the duodenum and proximal jejunum [3]. This depends on the physical state of the iron atom [38]. At physiological pH, iron exists in the oxidized, ferric (i.e. Fe^{3+}) state. To be absorbed, it must be in the ferrous (i.e. Fe^{2+}) state or bound by a protein such as heme [38]. In the intestinal lumen, ferrous iron is then transported across the apical membrane into the enterocytes by a protein called divalent metal cation transporter 1 (DMT1) [9]. In the enterocyte, iron is stored as ferritin or transported through the basolateral membrane into blood bound to ferroportin [3,9,36,38]. The transmembrane protein ferroportin is the only efflux route of cellular iron into circulation and is regulated almost exclusively by hepcidin levels [38,39]. Hepcidin binds ferroportin, causing internalization, degradation and favoring cellular iron to be stored as ferritin stores, hampering its absorption into the blood [40].

Cells with high metabolic rates, as in pregnancy, require more iron and are at greater risk of deterioration if iron deficiency is incurred [41]. Iron requirements during pregnancy increase dramatically, to sustain the increased demand of the growing fetus. Iron sufficiency is essential for oxygen delivery to the maternal- placental-fetal unit to support the increased oxygen consumption demand of pregnancy [42].

The human body has a total of about 3–4 g of iron, and this depends on gender, age, and nutritional habits [43]. Nearly 75 % of the total body iron is in the functional form mostly as hemoglobin in circulating RBCs and 15% as myoglobin [44]. The remaining 10% of body iron is stored in the liver, the reticulo-endothelial cells, and the bone marrow (33). A stored form of iron exists in the forms of two proteins, ferritin and hemosiderin [9].

Despite the increase in plasma volume, red cell mass, and hemoglobin levels during pregnancy, the increase in plasma volume is disproportionately greater than the increase in red cell mass or hemoglobin, causing dilutional anemia [45]. Pregnant women are highly prone to developing iron deficiency and iron deficiency anemia because of their high demand for nutrients, needs that can rarely be met through diet alone [3]. The total iron demand during pregnancy is the result of fetal iron deposition and the rate of deposition varies with the stage of pregnancy [46]. Most of the deposition occurs during the second and third trimesters of gestation which poses a higher risk of anemia [46].

Intermittent or daily iron and folic acid supplementation is currently recommended by WHO as part of antenatal care, to reduce the risk of low birth weight, maternal anemia and neural tube defects [10]. Prophylactic daily iron folate supplementation containing 30-60 mg of iron and 400 µg of folic acid is recommended by WHO as part of antenatal care with a higher dose preferred in settings where anemia in pregnant women is a severe public health problem (≥40%) [10]. This supplement is given to pregnant women starting immediately after conception [16]. Despite this recommendation, studies show iron supplementation in iron-sufficient individuals can result in health risks [14].

Folate also known as vitamin B9 or folic acid, is an essential vitamin required for RNA and DNA synthesis within the maturing erythrocyte [36]. Folates are coenzymes required for the synthesis of thymine and purines (i.e., adenine and guanine) and the conversion of homocysteine to methionine [31]. Deficient production of thymine, in particular, affects cells undergoing rapid division (e.g., bone marrow cells undergoing erythropoiesis) [44]. Humans are dependent on dietary intake to meet the daily requirement of 50 to 200 mg/day and increased amounts are required for lactating and

pregnant women [34]. Folate is absorbed from the upper small intestine and does not require any other element (i.e., intrinsic factor) to facilitate absorption [44]. After absorption, folate circulates through the liver, where it is stored [3]. A deficiency of folic acid results in megaloblastic anemia, red blood cells that are of larger size because of impaired DNA synthesis, which inhibits nuclear division.

2.2 Normal hematological changes during pregnancy

Pregnancy induces significant hematological changes to meet the increased metabolic demands and ensure an adequate supply of oxygen and nutrients to the developing fetus [47]. These changes are considered physiological adaptations and can affect almost all components of the hematological system, including plasma volume, red blood cells, white blood cells, and coagulation factors [36].

A significant hematological adaptation during pregnancy is the increase in plasma volume, which begins as early as the first trimester and peaks between the 30th and 34th weeks of gestation [48]. Plasma volume increases by 40% to 50% by term, a change driven by hormonal influences, particularly the renin-angiotensin-aldosterone system [49]. Activation of the RAAS system leads to increased plasma levels of aldosterone and subsequent salt and water retention in the distal tubule and collecting ducts [47]. This expansion serves to improve uteroplacental perfusion and maintain adequate blood flow to the fetus [50]. The increase in plasma volume leads to hemodilution, a phenomenon that results in lower concentrations of hemoglobin and hematocrit, a condition often referred to as physiological anemia of pregnancy [6]. Despite this relative anemia, there is an absolute increase in red blood cell mass by about 20% to 30% [51], which helps to meet the increased oxygen demands of both the mother and fetus.

Red blood cell production increases significantly during pregnancy to counterbalance the dilutional effect of increased plasma volume [52]. Iron requirements also rise sharply due to the need for increased erythropoiesis and fetal development, leading to a common recommendation for iron supplementation in pregnant women to prevent iron deficiency anemia [10]. Studies have shown that the total iron requirement during pregnancy is about 1000 mg, with approximately 500 mg used for the expansion of red blood cell mass and 300 mg for fetal and placental needs [53]. The physiological adaptations in red blood cell production also include changes in red blood cell morphology, such as increased cell size and altered membrane properties, which enhance the cells' ability to deliver oxygen effectively [54]. Additionally, there is an increase in 2,3-bisphosphoglycerate, which reduces hemoglobin's affinity for oxygen and facilitates oxygen release to fetal tissues [55].

Leukocytosis, or an increase in white blood cell count, is another common hematological change during pregnancy [56]. The total white blood cell count typically increases, and this increment is not indicative of infection but reflects a physiological response to the heightened inflammatory state associated with pregnancy and the body's preparation for the stress of labor and delivery [57]. In addition to quantitative changes, qualitative alterations in immune function also occur, aimed at maintaining a delicate balance between immune tolerance to the fetus and the mother's ability to defend against infections. This immune modulation includes a shift towards a Th2-dominated immune response, which favors humoral immunity and suppresses cell-mediated immunity to protect the fetus from rejection [58].

During pregnancy, there is a slight reduction in platelet count, a condition known as gestational thrombocytopenia, which occurs in about 7% to 10% of pregnancies [47,59]. This decrease is largely attributed to hemodilution, increased platelet turnover, and consumption due to placental sequestration and increased coagulation activity [60]. Despite the reduction in platelet count, platelet function remains largely unaltered, which ensures effective hemostasis during pregnancy and childbirth [61].

Pregnancy is associated with a hypercoagulable state, which serves to protect the mother from hemorrhage during delivery but also increases the risk of thromboembolic events [62]. This state is characterized by increased levels of coagulation factors, including fibrinogen, factors VII, VIII, IX, and X, and decreased activity of natural anticoagulants such as protein S and antithrombin [63]. The heightened coagulation potential is further supported by reduced fibrinolytic activity, ensuring rapid and efficient clot formation [47,64,65]. Prophylactic anticoagulation may be indicated in these high-risk groups to prevent complications such as deep vein thrombosis and pulmonary embolism [66].

2.3 The magnitude of iron deficiency anemia during pregnancy and its predictors

In 2019, the prevalence of anemia globally was 29.9% in women of reproductive age, equivalent to over half a billion women aged 15-49 years [67]. Prevalence was 29.6% in non-pregnant women of reproductive age, and 36.5% in pregnant women [68]. In a multicenter prospective cross-sectional study conducted in Australia, iron deficiency anemia prevalence in the first trimester of pregnancy is reported as 12%, increasing progressively to a higher percentage as the pregnancy advances to term (68% in the third trimester) [69].

A randomized controlled trial conducted in Norway showed a daily dose of 27 mg elemental iron, containing a heme component, given in the second half of pregnancy to help prevent depletion of maternal iron stores even after birth, in most women [70]. In a randomized controlled trial conducted in Israel, after pregnant women were supplied with 100 mg of elemental iron for five months, only 8.5% of participants had Hb level <10mg/dl and a higher prevalence of anemia was observed in the older women and women with higher parity [54].

In a randomized institution-based, double-masked study conducted in a Shantytown in Lima, Peru, after pregnant women were supplemented with 60 mg elemental iron, and 250 μg folic acid, hematologic changes were related to initial hemoglobin status and women with better initial Hb levels benefited much more than women with lower initial Hb level [71]. In this study women with anemia (hemoglobin <11 g/dl) were found to have steady increases in hemoglobin concentration through the course of pregnancy whereas women with relatively higher initial hemoglobin concentration showed a decremental trend during mid-pregnancy and then rising by 37–38 weeks of pregnancy. Despite the supplementation, women with poorer hematologic status, multiparous; and pregnant women who consumed fewer supplements were more likely to have anemia at the end of pregnancy [71]. It has also been reported that the prevalence of anemia within three months post-partum was significantly higher in pregnant women who did not take the recommended dose of iron folate supplementation [72], implying the role of IFA supplementation in post-birth maternal iron stores.

In pregnant women who were in their third trimester, reticulocyte counts, red blood cell distribution width and hemoglobin concentrations increased markedly four weeks after daily 200mg ferrous fumarate supplementation [73]. In a case-control study conducted in 2021, it was found that after participants were supplied with 100 mg daily oral ferrous fumarate, both ferritin and Hb levels increased two months post-supplementation [74]. Furthermore, in this study, gastrointestinal side

effects of the iron supplement were reported to be higher in the daily supplementation group than in intermittent supplementation. A cross-sectional study done in Indonesia found that the prevalence of anemia three months post iron supplementation was 34.3% (Hb<10.5g/dl). This study also depicted that low BMI during pregnancy has a significant association with maternal anemia and anemia tended to be more prevalent in the third trimester than second trimester [75].

A double-blind RCT study conducted in China in 2015 showed that prenatal iron supplementation reduced anemia, iron deficiency, and IDA in pregnant women [76]. However, in this study, most women and more than 45 % of neonates had iron deficiency, regardless of supplementation. A study conducted in Malaysia reported the prevalence of anemia in pregnant mothers as 57.4 %. In this study, anemia is reported to be significantly associated with being a grand-multigravida mother and late ANC booking [77]. In an institutional cohort study conducted on pregnant women in Nepal, all of the participants in the third trimester of pregnancy were anemic with Hg<11mg/dl [78] . In this study, women aged 17 to 24 years and multiparous women had higher odds of having anemia. This study also found that anemia in the third trimester of pregnancy is associated with low birth weight, preterm births, and neonatal intensive care unit stay.

A multicenter randomized controlled trial, conducted in Kenya reported the prevalence of IDA post supplementation is 34%. It is also reported in this study that prenatal IFA supplementation and consumption of heme-containing food help to decrease the incidence of iron deficiency anemia in pregnant women [79]. A multicenter prospective study conducted in Ghana, reported a higher prevalence of anemia during pregnancy [(54.4% (mild = 31.1%; moderate =23.1%; severe = 0.2%] [80]. In this study, microcytic (79.4%) and hypochromic (29.3%) anemia were most prevalent, and women with lower BMI, belonging to the AB blood group, poor dietary intake, maternal age below 19 years, not planning the pregnancy are reported more likely to be anemic, during pregnancy, particularly in the third trimester.

Several studies [81–83] conducted in Ethiopia had reported the prevalence of anemia as high, despite the universal supplementation strategy employed by the Ethiopian FMOH. This could be attributed to poor compliance, chronic co-morbid conditions, low-dose supplementation and nutritional deficiencies. Duration of IFA supplementation, tea consumption interfering with iron absorption, low maternal educational status (poor compliance) could impact the response to the IFA supplementation [84] .

In Ethiopia a study has found that only one in two pregnant women was supplemented with iron during their recent pregnancy [85]. The overall prevalence of anemia among pregnant women was 41 % of which 20% were moderately anemic, 18%, were mildly anemic, and were 3%, severely anemic [22]. An institution based follow-up study conducted in Mekelle city revealed that 48.5% of participants lacked adequate hemoglobin response, despite the IFA supplementation [23]. However, in this study prevalence of anemia and low hematocrit value decreased significantly after IFA supplementation. In this study one month of IFA supplementation reduced anemia in pregnant women from 25.5 % to 13.8 %. Normocytic hypochromic anemia is found to be the most common form of anemia among the study participants followed by normocytic normochromic anemia, morphologically atypical of iron deficiency anemia [23]. This prospective, hospital-based study also reported that IFA supplementation in pregnancy increased the hemoglobin content of RBCs [AOR: 1.02 (95% CI: 0.97- 1.07)]. A decrease in Hb concentration was also observed in pregnant women in a study conducted in Mecha woreda, Amhara region of Ethiopia with higher gravidity, age of the mother and increase in gestational age [86].

2.4 Iron supplementation and platelet parameters

The relationship between iron and thrombopoiesis is not well established [87]. In one study, platelet counts were increased when serum iron, iron saturation, ferritin and platelet count were decreased [88]. In this study, an inverse correlation between platelet distribution width and mean corpuscular volume was reported. Another study has shown there exists an inverse relationship between platelet counts, both mean platelet volume and iron saturation; and iron having an inhibitor effect on platelet counts [88]. A retrospective RCT reported that, in 8 weeks of continuous iron supplementation, the mean platelet counts decreased at 2-week treatment intervals [89]. It has also been reported that treatment of IDA with iron folate supplementation, results in a decrease in platelet count [90]. In this study a decrease in platelet count, an increase in platelet distribution width, and no change in mean platelet volume were observed before and after supplementation when IDA with normal platelet count was treated.

Iron deficiency is a common cause of reactive thrombocytosis. This finding is supported by clinical reports [91–93], meta-analysis studies [94,95] and animal studies [96,97]. Although the exact cause of thrombocytosis in iron deficiency is not fully understood, it is stipulated that the increase in erythropoietin in iron deficiency stimulates and upregulates the thrombopoietin receptors, and this in turn results in thrombocytosis [98].

2.5 Prenatal iron deficiency and birth outcomes

Studies have shown that IDA, especially in moderate and severe forms, is associated with adverse obstetric and birth outcomes, impacting maternal and fetal health. A systematic review and meta-analysis study showed that babies born to mothers with IDA had low birth weight and a significantly higher risk of low birth weight [(AOR 1.29(1.09 - 1.53)] and preterm birth [(AOR 1.21, 95% CI(1.13 - 1.30)] [18]. The risk of low birth weight and premature delivery was disproportionately higher if the anemia occurred during the first and second trimester of pregnancy than in the third trimester [99].

A retrospective cohort study conducted in China in 2022 reported that anemia of any degree is linked with an increased risk of maternal and fetal complications [100]. Iron supplementation during pregnancy is associated with an improvement in the birth weight of infants, and this is more pronounced in babies born to mothers suffering from anemia antepartum [100]. An institution-based study done in Nigeria showed that iron supplementation, despite being a good intervention in developing countries, is not sufficient to reduce the prevalence of anemia by the third trimester. This study underpinned the need to look beyond the approach and suggest the pros of food fortification and reducing the frequency of pregnancies [101].

3. CONCEPTUAL FRAMEWORK

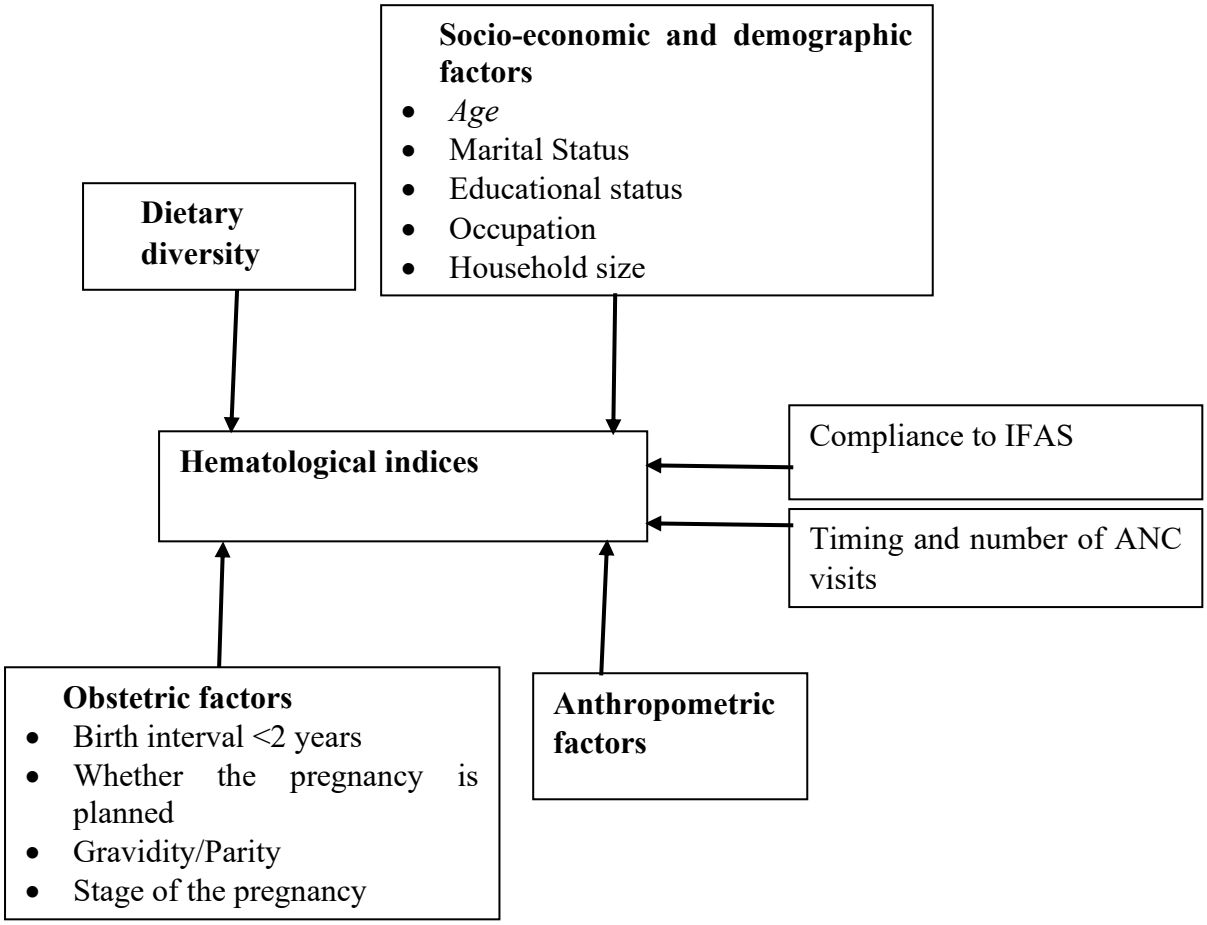


Figure 1: Conceptual framework to assess determinants and effects of IFAS on maternal hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa. (Adapted from research articles [23, 68, 117, 120].

4. RESEARCH HYPOTHESIS

Hypothesis 1: In pregnant women without anemia, prophylactic IFA supplementation would reduce the incidence of IDA.

Hypothesis 2: In pregnant women with mild and moderate anemia (Hb level 7mg/dl -10.9 mg/dl), therapeutic IFA supplementation would revert the hemoglobin level to normal.

5. OBJECTIVES

5.1 General Objective:

To assess iron- folate supplementation and associated factors on maternal hematological indices among pregnant women attending ante-natal care units in public hospitals in Addis Ababa, Ethiopia.

5.2 Specific Objectives:

To describe the effect of iron- folate supplementation on maternal red blood cell indices among pregnant women attending ANC unit in public hospitals in Addis Ababa.

To determine the effect of iron- folate supplementation on maternal platelet count among pregnant women attending ANC unit in public hospitals in Addis Ababa.

To assess the effect of iron- folate supplementation on maternal leukocyte count among pregnant women attending ANC unit in public hospitals in Addis Ababa.

To determine factors associated with iron folate supplementation among pregnant women attending ANC unit in public hospitals in Addis Ababa.

6. MATERIALS AND METHODS

6.1 Study period and setting

The study was conducted in selected Public Hospitals in Addis Ababa, the capital of Ethiopia, from May 1, 2023 to March 30, 2024. Among the twelve government hospitals, three hospitals namely, Zewditu Memorial hospital, St. Paul's Millennium Hospital Medical College and Menelik Hospital were selected by simple random sampling technique. Pregnant women whose weeks of pregnancy were identified by the last normal menstrual period technique (weeks of pregnancy calculated starting from the first day of the last normal menstrual period), attending ANC units in the aforementioned hospitals were the study participants. The city has a projected population of 3,603,000, and 49.97% are females, of whom 34.4% are in the reproductive age group, according to the 2019 CSA projection [102]. The city lies at an elevation of 2,355 meters above sea level.

6.2 Study Design

An institution-based retrospective cross-sectional study design was used in pregnant women attending Antenatal care units in the selected public hospitals in Addis Ababa, From May 1, 2023 to March 30, 2024.

6.3 Population

6.3.1 Source Population

All pregnant women above 18 years of age, who have ANC booking at the selected public Hospitals in Addis Ababa, Ethiopia.

6.3.2 Study population

All randomly selected pregnant women who were above 18 years of age, had ANC booking during the time of data collection at each selected respective public Hospital in Addis Ababa, Ethiopia.

6.4 Eligibility criteria

6.4.1 Inclusion Criteria

Women with singleton pregnancy, above 18 years of age, signed the written informed consent form, started IFAS with Hb level >7 mg/dl, had their ANC follow-up at the selected public hospitals during the time of the data collection in Addis Ababa, Ethiopia.

6.4.2 Exclusion Criteria

Women who had severe anemia initially and throughout the pregnancy (Hb<7 mg/dl), blood transfusion, smoking, comorbid conditions including but not limited to HTN, DM, CKD, known hematologic disorders, infections like helminthiasis, HIV and malaria.

6.5 Sample Size Determination, sampling technique and Procedure

6.5.1 Sample Size Determination

In designing this prospective follow-up study to assess the effect of iron folate supplementation on maternal red blood cell hemoglobin content (i.e., the primary outcome), we performed a detailed sample size calculation to ensure sufficient statistical power and representativeness of our sample.

Parameters for Sample Size Calculation:

1. Confidence Level: 95% ($za/2 = 1.96$)
2. Power: 80% ($z\beta = 0.84$)
3. Effect Size (Hb): 0.69
4. Relative Risk (RR) for Anemic: 0.45
5. Relative Risk (RR) for Non-anemic: 0.69

These parameters were informed by a prior study [103], which reported that:

- In pregnant women who took therapeutic iron- folate supplementation, the intervention resulted in a 55 % reduction of anemia and in those who took prophylactic iron folate supplementation, supplementation resulted in a 31% reduction in the risk of anemia.

Using STATA version 16 software and the aforementioned parameters, the initial sample size was calculated as: n=373. To account for potential non-response and ensure a robust sample, we adjusted for a 10% non-response rate:

$$n_{adjusted} = \frac{373 \times 10}{100} + 373 \quad = 410$$

Thus, the final sample size required for this study is 410. This sample size provides adequate power to detect significant differences in hemoglobin levels between anemic and non-anemic pregnant women following iron folate supplementation, ensuring the reliability and validity of our study findings.

6.5.2 Sampling technique and procedure

Based on previous hospital records, the total number of pregnant women who had their ANC follow-up at each selected hospital (in a month before the start of data collection) was obtained. This was followed by a proportional allocation of the number of ANC attendees to the respective hospitals. Using the ANC registry in the respective hospitals as a sampling frame, a systematic random sampling method was employed to select pregnant women who have ANC booking at the selected public hospital. Pregnant women getting ANC service at each selected public hospital during the data collection/study period, who met the inclusion criteria have had a random chance of being approached. Interviews were performed until the required sample size of 410 was achieved. Consent was secured verbally and in written form, before the start of the data collection.

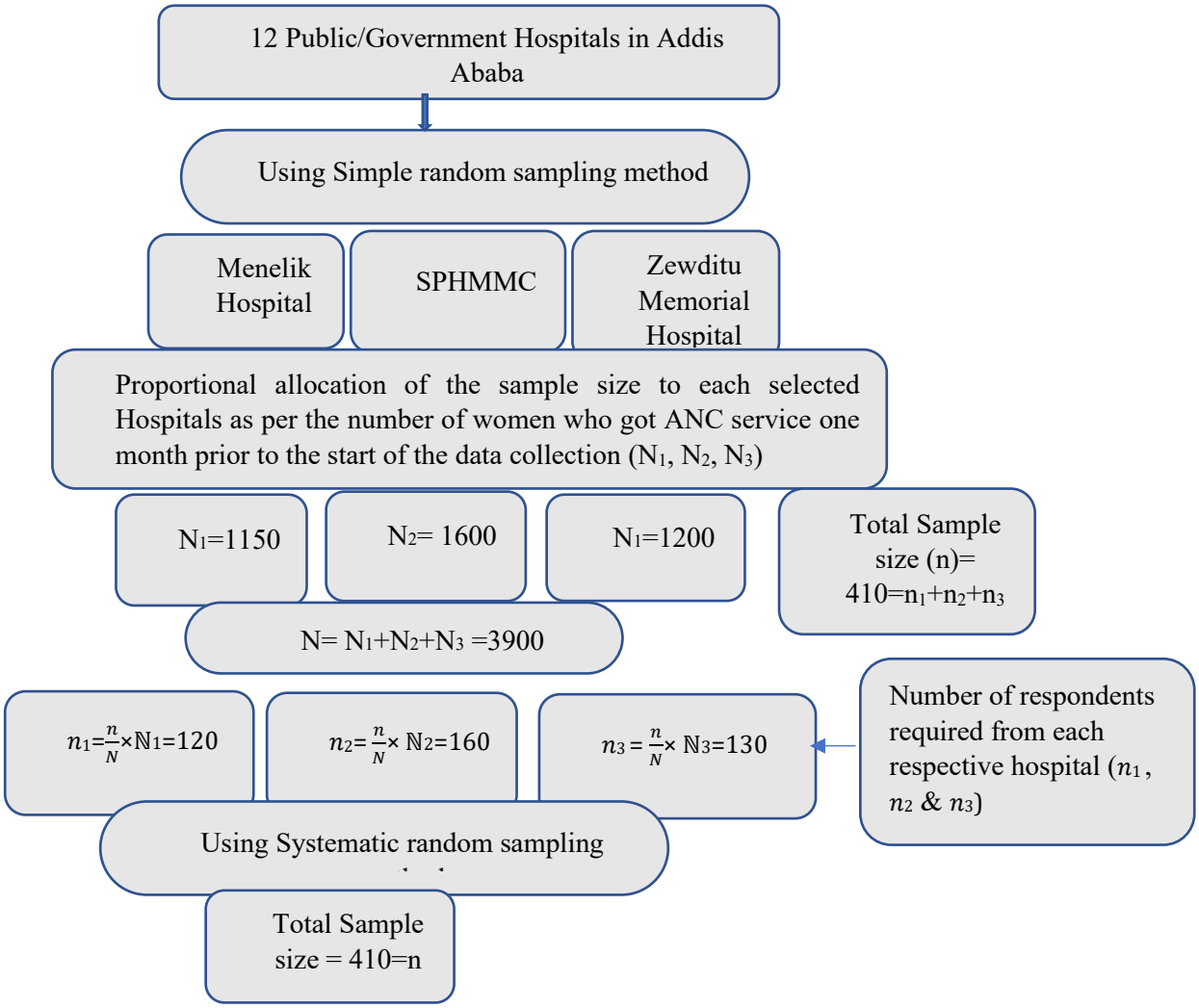


Figure 2: Sampling procedure to assess determinants and effects of IFA supplementation on maternal hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa.

6.6 Variables of the study

6.6.1 Dependent variable

Maternal hematological indices.

6.6.2 Independent variable

Sociodemographic factors (age, occupation, income, educational status, marital status, household size), MUAC, birth interval, parity, stage of the pregnancy, maternal blood group, timing of ANC booking, IFAS adherence, duration of intake of IFAS, planned or unplanned pregnancy, and dietary diversity score.

6.7 Operational definitions

Anemia was defined as a hemoglobin value of less than 11 g/dL [104]. Hemoglobin responses to the IFA supplementation were categorized as either adequate or inadequate [12,23]. The criteria for these categories were as follows:

Adequate Response: An increase in hemoglobin value by at least 1 g/dL after a minimum of one month of supplementation, indicative of iron deficiency [105,106].

Inadequate Response: A change in hemoglobin value of less than 1 g/dL after a minimum of one month of IFA supplementation. This response suggests the presence of functional iron deficiencies [23].

The normal reference range for hematological parameters, depicted bellow was used: MCH: 27–32 picogram (pg) per cell; MCHC: 31.5–36 g/dl; MCV: 80–100 fL; Hematocrit: 31–43 % ; RBC count: 3–4.5 $x10^{12}$ cells/L, WBC count: 4-15X 10^{9} cells/L and platelet count: 150-450$x10^{9}$/L [9]. Anemia was classified morphologically as macrocytic, microcytic, hypochromic and mixed types based on MCV and MCH and/or MCHC values [3]. The severity of anemia was classified as follows: mild anaemia (Hb: 10–10.9 g/dl), moderate anaemia (Hb: 7–9.9 g/dl), and severe anaemia (Hb < 7.0 g/dl) [10]. Microcytosis, macrocytosis and hypochromia were defined as MCV < 80 fl, MCV ≥ 101 fl and MCH < 27 pg respectively [108]. RDW greater than 15% was considered elevated [109]. Gestational ages were categorized as the first trimester (1–14 weeks), second trimester (15–28 weeks) and third trimester (29 and above), using WHO classification criteria. Pregnant women with a MUAC value of 23 - 33 cm were considered to have normal nutritional status; those with MUAC measurement < 23 cm were categorized as undernourished and those above 33 cm were categorized as obese [110,111]. Blood pressure measurement of systolic blood pressure ≥140 mmHg and diastolic blood pressure greater than ≥90 mmHg was considered elevated [112].

Compliance with the IFA supplement was assessed based on the pill count and self-report method. Women who took 70% or more of the IFA tablets, equivalent to taking at least 5 days a week throughout the study period, were considered adherent [113], using recording, self-reporting, pill counting and checking their cards. Otherwise, participants were considered non-adherent and excluded from the study.

Dietary intake was assessed using a food frequency questionnaire using the minimum dietary diversity for women (MDD-W), adopted by the food and agricultural organization of the United Nations. The minimum dietary diversity for women (MDD-W) is a population-level indicator of diet diversity validated for women aged 15-49 years. It is a dichotomous indicator based on 10 food groups consumed locally (in this case in Ethiopia) and is considered the standard for measuring population-level dietary diversity in women of reproductive age [114] (Table 9). Pregnant women who consumed five or more food items in the last 24 hours, out of the 10 food groups, were considered to have adequate dietary diversity, otherwise they were considered to have poor dietary diversity.

6.8 Data Collection Procedures, tools and quality control

6.8.1 Data collection tools

A structured questionnaire was prepared in English and Amharic languages. The Amharic version was filled by the participants if they were able to read and write in Amharic language, otherwise, the data collector completed the questionnaire by asking the respondents. The response was translated back into the English language. The questions explored and addressed the respondent's sociodemographic profiles, data on parity/gravidity, intake of IFAS and dietary diversity.

6.8.2 Data collection procedure and quality control

Pregnant women who met the inclusion criteria and were prescribed 60 mg elemental iron plus 400 µg folic acid oral tablets once daily if non-anemic and 120 mg elemental iron plus 800 µg folic acid once daily if anemic, were approached. The hematological profile of the participants was performed two times i.e., at the first ANC visit (baseline, before the start of the IFA supplementation) and after intake of iron folic acid tablets, at the endpoint of the follow-up. All the respondents were taking similar iron-folic acid tablets throughout the follow-up period.

Five ml of venous blood sample was collected while the respondents were comfortably seated, using iron-free heparinized test tubes by professional nurses, from respondents in each respective hospital. Hematological analysis was then performed at each respective hospital. The procedure was

performed for assessment of the hematological profiles, at baseline and the end point (after at least 4 weeks of IFAS supplementation). Standard precaution measures were strictly followed while taking blood samples and after the blood sample was taken. Hematological analysis was performed using Cell-Dyn1800 (Abbott Laboratories Diagnostics Division, USA) and Mindray Auto Hematology analyzer (Mindray Bio-Medical Electronics Co. LTD, China). While performing measurements, quality control measures as per the manufacturer's recommendations were strictly obeyed.

Mid-upper arm circumference was measured using WHO recommendation using non stretchable measuring tape, to assess the participants' nutritional status. Blood pressure was measured at the endpoint after iron folate supplementation, in both arms, in a position where the brachial artery at the antecubital fossa, is at the heart level. Participants were asked if they took tea and or caffeine. Participants were seated, with feet supported, for 5 minutes before blood pressure was measured. Blood pressure with the higher value taken from the two arms was recorded.

Data was collected by the investigators assigned at each chosen hospital. To ensure the completeness, accuracy and consistency of data collection and for the investigators to have a common understanding of how to approach the participants, training was given before the start of data-collection by the main investigator. On-site supervision was carried weekly, by the principal investigator. A pretest was done in Abebech Gobena MCH Hospital, Addis Ababa, on 10% of the sample to check for the accuracy of responses, language clarity, and appropriateness of the tools. Following the pretest, some adjustments were performed, including typing errors being fixed, data collectors being reoriented, and questionnaires being rearranged.

6.9 Data processing and analysis

The completeness and consistency of the data was checked. The data was then entered into epi data version 4.6 software. After the data was edited & coded, it was exported to SPSS version 27 software for further analysis. Descriptive statistics was used to describe the profile of the study participants and to determine the hematological indices of the pregnant women enrolled in the study. Ninety-five percent CI and corresponding P values < 0.05 were used to declare statistical significance. The Hosmer–Lemeshow test and Shapiro-wilk test were used. Paired sample t-tests and Wilcoxon signed-rank tests were also performed to detect statistically significant deviations between results before and after IFA supplementation. A logistic regression model was fitted to identify the associated variables.

6.10 Ethical considerations

Ethical approval was obtained from the research and ethical review committee of the department of Physiology, College of Health Sciences, AAU. Additional ethical approval was also obtained from Addis Ababa city health bureau and SPHMMC with reference numbers A/A/12240/227 and Pm23/1024 respectively. Following receipt of the letter of approval, the investigator proceeded to the respective chosen hospitals to receive an authorization letter for the collection of data from the respective hospitals. The respondents were informed of the aim of the study, given the chance to withdraw from the study anytime they felt uncomfortable. Written informed consent was also obtained from each respondent. To maintain privacy and confidentiality, the respondent's name was replaced by codes while the information was used only for the objectives of the study.

6.11 Dissemination of the result

The results of the findings will be submitted and communicated to the department of Physiology, College of Health sciences, Addis Ababa University. Addis Ababa City Health Bureau, and Ministry of Health Ethiopia, to acquaint them with the study's findings and recommendations. The document can be used to establish baseline reference data for possible future interventions. The findings will be presented in scientific workshops, conferences and will also be published in scientific journals.

7. RESULT

7.1 Socio-demographic characteristics of the study participants

Four-hundred ten subjects participated in the study with a response rate of 100%. The age of the study subjects ranged from 19 to 43 years with a median age of 30 and IQR (34-26) years. The majority of the respondents attended secondary school 261 (63.7%) while 395 (96.3%) of the respondents were permanent urban residents. Unemployed respondents accounted for 274 (66.8%) of the participants. Most of the respondents were married (90.5%). The median (IQR) of the participants' average monthly income was 12000 (23000 - 7500) Ethiopian birr respectively (**Table 1**).

Table 1: Socio-demographic characteristics of pregnant women attending antenatal care units in selected public hospitals in Addis Ababa, Ethiopia, from May 1 to March 30, 2023 (n=410)

Variables	Category	Frequency	Percentage
Age in years*	<20	4	1
	20-29	189	46.1
	30-39	209	51
	>40	8	2
Residency	Urban	395	96.3
	Rural	15	3.7
Occupation	Employed	136	33.2
	Unemployed	274	66.8
Marital status	Unmarried	39	9.5
	Married	371	90.5
Household Size	1-5	384	93.7
	>5	26	6.3
Educational Status	No formal education	11	2.7
	Attended Primary school	45	11
	Attended Secondary school	261	63.7
	Higher education	93	22.7

*Age category was adopted from a previous research article [112].

7.2 Clinical characteristics of the study participants

Most of the participants (58.8%) were in their third trimester of pregnancy (i.e., at the end of the follow-up) with multiparity (54.4%) and more than half of the participants started ANC booking in the early first trimester. The majority of the respondents (80.5%) had a birth interval of more than 2 years. Two hundred eighty-five respondents reported that their pregnancy was planned and more than half of respondents had been taking IFAS for ≥ 2 months. The most common time for IFAS and coffee/tea intake was after meals (53.7% & 71% respectively). Over half of the respondents (56.8%) had adequate MDD-W. About Three fourth of the participants had MUAC measurements within the normal range of 23-33 cm (**Table 2)**.

Table 2: Clinical Characteristics of Pregnant Women Attending Antenatal Care Units in selected Public Hospitals in Addis Ababa (n=410)

Variables	Category	Frequency	Percentage
Pregnancy stage	Second trimester	169	41.2
	Third trimester	241	58.8
Parity	≤ 1	84	20.5
	2-4	223	54.4
	≥ 5 .	103	25.1
Duration of IFAS	≥ 2 months	206	50.3
	1 - 2 months	204	49.7
IFAS Intake	After meal	220	53.7
	Before meal	126	30.7
	No time preference	64	15.6
Birth interval	<2 years	80	19.5
	>=2 years	330	80.5
MDD-W*	Adequate	233	56.8
	Inadequate	177	43.2
MUAC**	<23 cm	69	16.8
	23 – 33 cm	311	75.9
	>33 cm	30	7.3

Continued next page

ANC booking ***	Early First Trimester	222	54.15
	Late First Trimester	188	45.85
coffee/tea intake	After meal	291	71
	Before meal	52	12.7
	No time preference	67	16.3
pregnancy planned or not	Yes	285	69.5
	no	125	30.5

*MDD-W= Minimum Dietary Diversity for Women, **MUAC: Mid Upper Arm Circumference, adopted from a research article [111].

***Early first trimester: < 7 weeks after conception, late first trimester ≥ 7 weeks after conception

7.3 Hematological responses to IFA supplementation

Adequate Hb response, an increase in hemoglobin level by 1g/dl and above, was found in 40.7 % of the respondents taking IFA. Before IFA supplementation, more than one-third of the pregnant women (39.3%) were diagnosed with anemia (Hb<11.0 g/dl). After supplementation, this dropped to 17.3 % (p = 0.001). Sixty-three participants (15.3%) who were anemic before IFAS initiation, also remained anemic after supplementation. Eight pregnant women, with normal baseline hemoglobin levels developed anemia despite intake of IFAS.

A large proportion of the pregnant women had low MCHC (36.6%), MCV (35.4%), and MCH (35.6 %), on their first visit at baseline and a significant difference was observed after IFA supplementation (25.9 %, 23.9 %, and 25.4% respectively) (P<0.001). Only 0.7% and 3.9% of the respondents had low WBC count and platelet count respectively. This changed to 1% and 5.9% respectively, after IFA supplementation (p =0.442 and 0.863 respectively). Also, sixty-one (14.9%) of the pregnant women who took prophylactic iron- folate supplementation developed preeclampsia after intake of IFAS (Bp ≥ 140/90) (**Table 3**).

Table 3: Proportions of pregnant women with an abnormal hematological profile before and after IFA supplementation at antenatal care units in selected public hospitals in Addis Ababa (*NB: The baseline data was collected retrospectively from ANC recordings while the end line was recorded prospectively.)

Abnormal parameters		Baseline: n (%)	End point: n (%)	P-value
Low Hb (<11 g/dl)		161(39.9)	71(17.3)	0.001
Low HCT (<31 %)		92(22.4)	35(8.5)	0.001
Low MCV (<80 fl)		145(35.4)	98(23.9)	0.218
High MCV (>100 fl)		7(1.7)	6(1.7)	0.622
Low MCH (<27 pg/cell)		146(35.6)	104(25.4)	0.001
Low MCHC (<32 g/dl)		150(36.6)	106(25.9)	0.001
High RDW (≥15 %)		100(24.4)	75(13.9)	0001
Low Platelet (<150 × 10^3 cells /µl)		16(3.9)	24(5.9)	0.056
Blood pressure	Systolic (≥140 mmHg)	0	61(14.9)	0.001
	Diastolic (≥90 mmHg)	0	54(13.2)	0.001

The mean hemoglobin level in anemic respondents at the baseline and end line, respectively, was 10.3 g/dl and 11.2 g/dl (8.7 % increment, (P = 0.001)), while in non-anemic respondents, it changed from 12.3 g/dl to 12.9 g/dl (4.9 % increment, P=0.001). The mean hematocrit value was also changed from 31.8 % to 33.9 % (6.6 % increment) in anemic respondents (P=0.001) and from 37.1 % to 37.9 % in non-anemic respondents (2.2 % increment, P= 0.001) (Table 4).

The median WBC count in anemic respondents, before supplementation was 8,300 per mm3 of blood with an interquartile range (IQR) of (7,400 - 8,600 per mm3 of blood) and after supplementation, this was changed into 8,400 per mm3 of blood with an interquartile range (IQR) of (7,700 - 9,800 per mm3 of blood).

Table 4: Changes in the mean values of hematological profiles after IFA supplementation in non-anemic pregnant women attending antenatal care units in Addis Ababa Public Hospitals.

Parameters	Mean ±SD, baseline	Mean ±SD, endpoint	Mean difference (95% CI)	% Change	p-value
Hb (g/dl)	12.30±1.20	12.86±1.14	0.47(0.34-0.61)	3.88	0.001
HCT (%)	37.10±3.25	37.84±3.00	0.74(0.31-1.17)	1.99	0.001
RBC (10^6 /µl)	4.51±0.43	4.70±0.54	0.18(0.13-0.23)	4.21	0.001
MCV (fl)	84.50±7.31	87.81±6.49	3.36(2.36-4.35)	3.92	0.001
MCH (pg/cell)	30.10±3.56	30.20±2.55	0.10(-0.38-0.58)	0.33	0.687
MCHC (g/dl)	33.29±2.06	34.33±1.37	1.04 (0.79- 1.30	3.12	0.001
RDW (%)	13.55±1.42	13.88±1.09	0.33(0.13-0.53	2.44	0.001
WBC (10^3 / µl)	8.19±1.83	7.98±1.91	-0.20(-0.4-0.01)	-2.56	0.059
PLT (10^3 / µl)	274.90±56.3	254.29±59.07	-20.60(-26.7-(-14.5))	-7.49	0.001

SD: Standard deviations, CI: Confidence interval, PLT= platelet count, HCT= hematocrit, RBC= red blood cells, MCV= mean cell volume, MCH=mean cell hemoglobin, MCHC= mean cell hemoglobin concentration, RDW= red cell distribution width, WBC= white blood cells

In non-anemic participants, the median WBC count before supplementation was 8,300 per mm³ of blood with an interquartile range (IQR) of 1,200 (7,400 - 8,600 per mm³ of blood) and showed a slight increase after supplementation; into 8,400 per mm³ of blood with an interquartile range (IQR) of 2,100 (7,700 - 9,800 per mm³ of blood).

Table 5: Changes in the mean values of hematological profiles after IFA supplementation in anemic pregnant women attending antenatal care units in Addis Ababa public hospitals.

Parameters	Mean ±SD, baseline	Mean ±SD, endpoint	Mean difference (95% CI)	% Change	p-value
Hb (g/dl)	10.28±3.36	11.23 ± 0.75	0.95(0.8-1.1)	9.24	0.001
HCT (%)	31.83±2.29	33.89±2.38	2.10(1.7-2.5)	6.47	0.001
RBC (10^6/µl)	4.05±0.37	4.23±0.39	0.18(0.1-0.3)	4.44	0.001
MCV (fl)	83.04±8.11	85.40±8.25	2.35(1.27-3.40)	2.84	0.001
MCH (pg/cell)	27.35±1.93	28.44±1.94	1.09(0.70- 1.5)	3.99	0.001
MCHC (g/dl)	30.42±1.89	30.95±2.44	0.53(0.32-0.73)	1.74	0.001
RDW (%)	15.16±1.21	14.14±0.91	-1(-1.3-(-0.8))	-6.73	0.001
WBC (10^3 / µl)	8.15±0.84	8.32±1.14	0.2(-0.01-0.35)	2.09	0.065
PLT (10^3 / µl)	293.30±37.01	285.40±37.83	7.91(1.8-13.9)	-2.69	0.01

PLT= platelet count, HCT= hematocrit, RBC= red blood cells, MCV= mean cell volume, MCH=mean cell hemoglobin, MCHC= mean cell hemoglobin concentration, RDW= red cell distribution width, WBC= white blood cells

The median platelet count, in anemic respondents, at baseline was 300,000 per mm³ of blood with an interquartile range (IQR) of 75,000 (250,000 - 310,000 per mm³ of blood) and showed a slight difference after supplementation i.e., 295,000 per mm³ of blood with an interquartile range (IQR) of 30,000 (275,000 - 305,000 per mm³ of blood). Pre-supplementation median platelet count in non-anemic respondents was 250,000 per microliter (µL) of blood with an interquartile range (IQR) of 82,000 (220,000 - 302,000 per µL of blood) and changed into 240,000 per µL of blood with an interquartile range (IQR) of 85,000 (225,000 - 310,000 per µL of blood post supplementation.

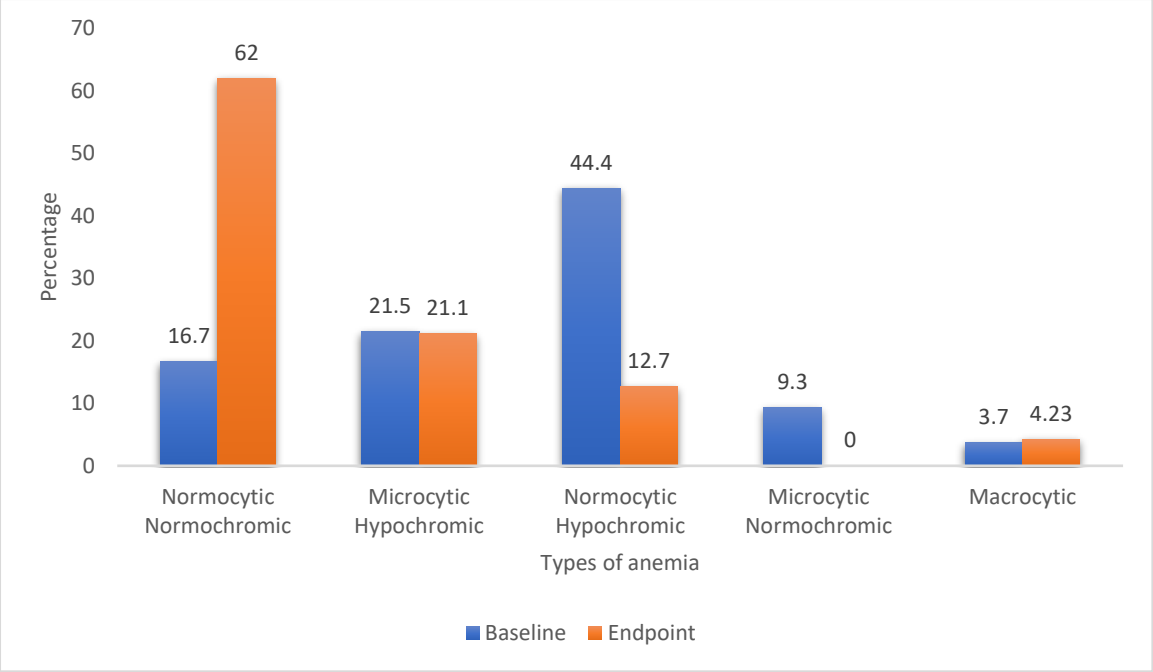


Figure 3: Anemia classifications based on red blood cell indices in pregnant women attending ante natal care units in Addis Ababa Public Hospitals.

7.4 Anemia morphology and classification

Of the 161 respondents diagnosed with anemia at baseline, 44.4 % (78/161) were morphologically mixed type (normocytic hypochromic anemia) with normal MCV (80–100 fl) and low MCHC (<32 g/dl). Only 35 (21.5%) of them were microcytic hypochromic, typical of iron deficiency anemia, with low MCV (<80 fl) and low MCHC and MCH. The remaining were 16.7% (27 out of 161) normocytic normochromic anemia, 3.7% (6/161) macrocytic hypochromic anemia and microcytic normochromic 9.3 % (15/161). At the endpoint, 44 (62%), 15 (21.1%), and 9 (12.7%) of the respondents had normocytic normochromic anemia, microcytic hypochromic anemia and normocytic hypochromic, respectively (Table 6, Figure 3). Initially at baseline, of the 161 respondents who were diagnosed with anemia, 140 of them had mild anemia, and the rest 21 respondents had anemia of moderate degree. After the follow-up, 65 respondents had mild anemia while only six respondents had anemia of moderate degree.

Table 6: Anemia classifications based on red blood cell indices in pregnant women attending antenatal care units in Addis Ababa Public Hospitals

Anemia morphology	Baseline n (%)	Endpoint n (%)
Normocytic normochromic	27(16.7)	44(62)
Microcytic hypochromic	35(21.7)	15(21.12)
Macrocytic	6(3.7)	3(4.23)
Normocytic hypochromic	78(44.4)	9(12.7)
Microcytic Normochromic	15(9.3)	0
Total	161 (100%)	71 (100%)

n= total number of respondents with anemia at baseline or endpoint.

7.5 Factors associated with hemoglobin response to IFA supplementation

Variables that were found to have corresponding p-value less than 0.25 in bivariate logistic regression, and hence entered into multivariable logistic regression analysis, were; marital status, planned or unplanned pregnancy, parity, MDD-W, duration of IFAS intake, the timing of ANC booking, experiencing side effects of IFAS and the number of tablet intake per week (intermittent intake or continuously all days of the week for the duration of the supplementation).

Table 7: Multivariable binary logistic regression on factors associated with hemoglobin response to iron folate supplementation among pregnant women attending antenatal care units in Addis Ababa public Hospitals

Variables	Response to IFA supplementation		COR (95%CI)	AOR (95%CI)	P value
	Adequate n (%)	Inadequate n (%)			
Marital Status					
Married	158	213	2.5(1.1– 5.4)	1.8(0.7-4.4)	0.23
Single	9	30	1	1	
Pregnancy					
Intended	135	150	2.6(2.64-4.16)	1.6(1.1– 3.5)	0.15
Unintended	32	93	1	1	
Parity					
< 2	48	36	2.3(1.4 – 3.7)	2.4(1.4– 4.2)	0.003
≥ 2	119	207	1	1	
Timing of ANC Booking					
≤ 7 Weeks	119	103	3.4(2.2 – 5.13)	3.9(2.4-6.6)	0.001
> 7 Weeks	48	140	1	1	

Continued next page

Duration of IFAS intake					
>=2 months	110	96	2.95(1.96– 4.5)	2.6(1.6-4.2)	0.001
1 - 2 months	57	147	1	1	
Side effects from IFAS					
Yes	81	159	0.49(0.3-0.7)	0.5(0.3-0.8)	0.007
No	86	84	1	1	
Tablet intake per week					
>5 tabs	142	155	3.2(1.96-5.30)	2.3(1.3- 4.1)	0.005
<=5 tabs	25	88	1	1	
MDD-W					
Adequate	147	30	2.55(1.7 – 3.8)	3.4(2.1-5.6)	0.001
Inadequate	96	137	1	1	

ANC: Antenatal Care, AOR: Adjusted odds ratio, COR: CI: Confidence interval, Crude odds ratio, IFAS: Iron folic acid supplementation, MDD-W: minimum dietary diversity for women

Among all the variables entered into multivariable logistic regression analysis, MDD-W, timing of ANC booking, duration of IFAS intake, average number of tablet intake per week, parity and experiencing IFA side effects showed statistically significant association with the main outcome variable (adequate Hb response after supplementation), after adjusting for confounding (Table 7).

Respondents who took IFA tablets for more than two months were 2.6 times more likely to have adequate hemoglobin response than respondents who took them 1-2 months (AOR =2.6, 95% CI (1.6-4.2). Also, pregnant women who reported they had side effects from IFA intake, are less likely to have an adequate response to IFA supplementation compared to those who do not experience side effects. (AOR= 0.5, 95% CI= 0.32-0.84). Furthermore, pregnant women who are adherent to IFAS are 2.28 times more likely to have adequate hemoglobin response to iron folate supplementation than those non-adherent respondents (AOR= 2.28, 95% CI =1.28- 4.1). This study has also found that primiparous respondents were 2.4 times more likely to have adequate hemoglobin response to iron folate supplementation than multiparous respondents (AOR= 2.4, 95% CI (1.4– 4.2)) (Table 7).

Pregnant women with adequate minimum dietary diversity for women (MDD-W) were 3.4 times more likely to have adequate hemoglobin response than those with inadequate MDD-W (AOR= 3.4, 95% CI=2.12-5.56). Additionally, pregnant women who booked for antenatal care at the health facility in the first seven weeks of the first trimester, were 3.9 times more likely to have adequate hemoglobin response to iron-folate supplementation than respondents who booked in later gestational weeks (AOR=3.9, AOR=2.41-6.56) (Table 7).

8. DISCUSSION

The study revealed that adequate hemoglobin response to iron-folate supplementation is present in only 40.7 %, [95% CI: 35.9% - 45.5 %)] of the respondents. This result is in line with a study done in Jordan (43.1%) [115] but lower than a study conducted in Mekelle, Ethiopia 48.5 [23], and a study of an analysis of five RCTs [106]. The difference could be due to differences in geographical location that can influence the body's iron metabolism and the overall effectiveness of supplementation programs. The higher altitude of Addis Ababa could necessitate greater physiological adaptations, thereby impacting the hematological response to iron-folate supplementation differently compared to Mekelle. According to other studies, oral iron therapy could increase hemoglobin levels, with near-maximal response rates achievable by day 28 post supplementation. A ≥1.0-g/dL hemoglobin increase after 2 weeks of oral iron therapy is an accurate predictor of subsequent hemoglobin responses at 6-8 weeks [106].

In this study, the percent changes of hematological indices after iron-folate supplementation, especially the red blood cell indices, is higher among anemic respondents than their non-anemic counterparts. Respondents with baseline hemoglobin level < 11g/dl had elevated RDW, which is higher than those respondents with ≥11g/dl hemoglobin level (Table 3). This result is consistent with previous studies [23,73,116,117]. The high RDW value in anemic respondents could be attributed to stimulated erythropoiesis [118]. Low oxygen levels due to anemia trigger a compensatory mechanism to increase red blood cell production and this stimulation of erythropoiesis in turn can lead to anisocytosis; an early sign of iron deficiency anemia [119].

In this study, the prevalence of anemia at the endpoint of the follow-up (after respondents completed intake of IFAS) is 17.3% (95% CI 13.8 – 21.8) (Figure 3). This is in line with a study done in Iran [120]. This finding is higher than a study conducted in Addis Ababa [121], but lower than a systematic review and meta-analysis study [81]. The discrepancy with the study in Addis Ababa could be because of lack of representativeness of the study as it was conducted in a hospital, as opposed to our study which is conducted in a multi-center setting. The difference with the systematic review and meta-analysis study might be because it included studies from various regions with differing dietary patterns and nutritional statuses, which could lead to an overall higher prevalence of anemia.

At baseline, about one-third of the anemic respondents had hypochromic anemia. The proportion of anemic respondents with hypochromia was significantly reduced at the endpoint of the follow-up, after iron folate supplementation (Table 3). Hypochromia is indicative of ineffective hemoglobin production in erythrocytes secondary to functional iron deficiency [122]. Our result is consistent with previous studies [23,117,121]. At the endpoint of the follow-up, normocytic normochromic anemia is found to be the most common form of anemia present in this study participants. Similar findings have been reported in previous studies [99,123]. In one study, microcytic normochromic anemia was the most common form reported [121]. The etiology of normocytic normochromic anemia could be hypo proliferative (i.e., corrected reticulocyte count <2%) or hyperproliferative (i.e., corrected reticulocyte count >2%) [124]. In iron deficiency anemia, the reticulocyte count could be normal or low [125]. Both anemia of chronic illness and IDA could be the cause of normocytic normochromic anemia, but the latter usually manifests with low MCV as opposed to the former [124].

The most common red blood cell alterations in this study, at the endpoint, were normocytic normochromic (62 %) and microcytic hypochromic (21.12 %) **(Table 6)**, which could be indicative of iron deficiency [108]. Normocytic normochromic anemia is a type of anemia in which the circulating red blood cells (RBCs) are of the same size (normocytic) and have a normal red color (normochromic) [124]. Normocytic normochromic red cell morphology with hemoglobin concentration less than 11 g/dl could be because of the physiological anemia of pregnancy [108]. Also, microcytic hypochromia or normocytic hypochromia with hemoglobin level < 11g/dl could be secondary to iron deficiency anemia [108]. In our study, normocytic hypochromia at baseline occurred in seventy-eight respondents while it occurred in only twelve respondents at the endpoint **(Table 6)**. It is caused by decreased iron reserves in the body which could be secondary to decreased iron in the diet, poor absorption of iron from the gut, blood loss, and increased demand for iron due to pregnancy [126]. Also, in this study 3.7 % of the anemic respondents had macrocytic anemia (MCV > 100, hemoglobin < 11g/dl) initially and at endpoint, only three cases of anemia were of macrocytic type. Macrocytic anemia is because of the deficiency of folic acid and vitamin B12 [127]. During pregnancy, there is an increase in mean corpuscular volume of about 4 fl on average in an iron replete women and this does not suggest any deficiency of vitamins B12 and folate [56].

In this study, microcytosis occurred in 23.9 % of respondents while hypochromia occurred in 25.4 % of respondents. Studies have shown that in iron deficiency anemia, hypochromia occurs more often than microcytosis [99–101]. In a five-year retrospective study, it has been shown that hypochromic anemia occurred in nearly 90% of patients with IDA cases while microcytosis was found only in 53% of iron deficiency anemia cases [128]. Other studies have also reported similar findings [129,130].

Baseline RDW value was higher in anemic compared to non-anemic respondents (mean 15.16% vs. 13.55 %). This finding is supported by prior studies that reported a higher RDW value in anemic than in non-anemic respondents [131–133]. The rise in RDW is because of morphologic changes that occur in iron deficiency anemia resulting in a microcytic population of cells appearing in the blood [134,135]. Furthermore, in our study, RDW showed a decreasing trend after iron folate supplementation in anemic respondents (mean 14.14%, $p<0.001$). This result is in line with previous studies that reported a decrease in RDW values when iron deficiency anemia is corrected [136]. However, this finding contradicts the result of a study conducted in Turkey which reported a rise in the RDW values right after the initiation of iron supplementation [137]. The difference could be due to differences in the baseline severity of anemia; as in our study severe forms of anemia were excluded, leading to a decrease in RDW over time. The Turkish study included respondents who had more severe iron deficiency anemia, resulting in a more pronounced initial variability in red blood cell size as the bone marrow begin to respond to supplementation.

Red blood cell count showed significant changes in both anemic and non-anemic respondents, at the endpoint, and the percent change in RBC was higher in anemic respondents than non-anemic (Table 4). This study is consistent with a study conducted in Mekelle, Ethiopia [23], but higher than a study conducted in Kakamega, Kenya [117]. The difference could be due to the elevation difference where the study participants reside, as the city where the latter study was conducted is located 5,100 feet above sea level compared to Addis Ababa, located at 7,726 feet above sea level, where this study is conducted. Residents at high altitudes cope with decreasing inspiratory oxygen partial pressure by stimulating erythropoiesis [3]. Long-term adaptation to high altitude enables healthy individuals to maintain their iron stores within the physiological range despite elevated requirements for erythropoiesis, but in vulnerable populations with increased iron demand like pregnant women, iron stores are less likely to be replenished quickly when living at high altitude [138].

There were no significant changes observed in the number of white blood cell counts in either anemic or non-anemic respondents at the endpoint. A similar finding has been reported in a study

by Belay et al [23]. However, a study by conducted in Poland has reported that iron folate supplementation has the effect of increasing white blood cell counts [139]. Significant change in WBC has also been reported after iron folate supplementation [121]. Leukocytosis, a common finding during pregnancy, is due to the physiologic stress induced by the pregnancy state [56]. Hormones such as estrogen and cortisol, elevated during pregnancy, also stimulate the bone marrow to produce more WBCs. White blood cell count could also decrease along with other hematological indices, in conditions like aplastic anemia, bone marrow failure, autoimmune disorders, and nutritional deficiencies like folate and vitamin B12 [9,140,141]. Additionally, WBC count could increase due to pathologic conditions like infections which are common during pregnancy and in certain malignancies like leukemias, lymphomas and multiple myeloma [9].

Platelet count decreased significantly at the endpoint of the follow-up, in both anemic and non-anemic respondents (Table 4 & Table 5). As the majority of the participants of the study were in the third trimester, at the endpoint, this could have resulted from hemodilution caused by the increased plasma volume and accelerating platelet clearance in later gestation [142]. Platelet counts in pregnant women begin to decrease in the mid-second to the third trimester and continue to decrease until the time of delivery [90]. Iron supplementation has been shown to decrease platelet count [89]. In our study, 24 (5.9%) of the respondents had thrombocytopenia at the end of the follow -up. The value of this result is consistent with previous studies [89,143], but lower than other studies [144], [123], 145].

Sixty-one (14.8%) respondents who initially took prophylactic iron folate supplementation with normal levels of hemoglobin and blood pressure developed hypertension de novo in later gestation. Studies have shown that iron-replete pregnant women taking iron folate supplementation have higher odds of gestational hypertension as compared to iron-depleted pregnant women [112]. According to other studies, higher maternal early pregnancy serum ferritin concentrations are associated with higher systolic and diastolic blood pressure throughout pregnancy but no consistent association is present between maternal iron status in early pregnancy with gestational hemodynamic adaptations or the risks of gestational hypertensive disorders [146]. Several other studies have shown that iron folate supplementation in iron-replete pregnant women poses a risk of preeclampsia [147–149]. The Physiological mechanism by which the high level of iron in the body causes preeclampsia involves reactive oxygen species generated by iron-dependent lipid peroxidation [150]. These ROS increase oxidative stress and induce the production of pro-inflammatory cytokines that contribute to endothelial dysfunction, a defining feature of preeclampsia [151].

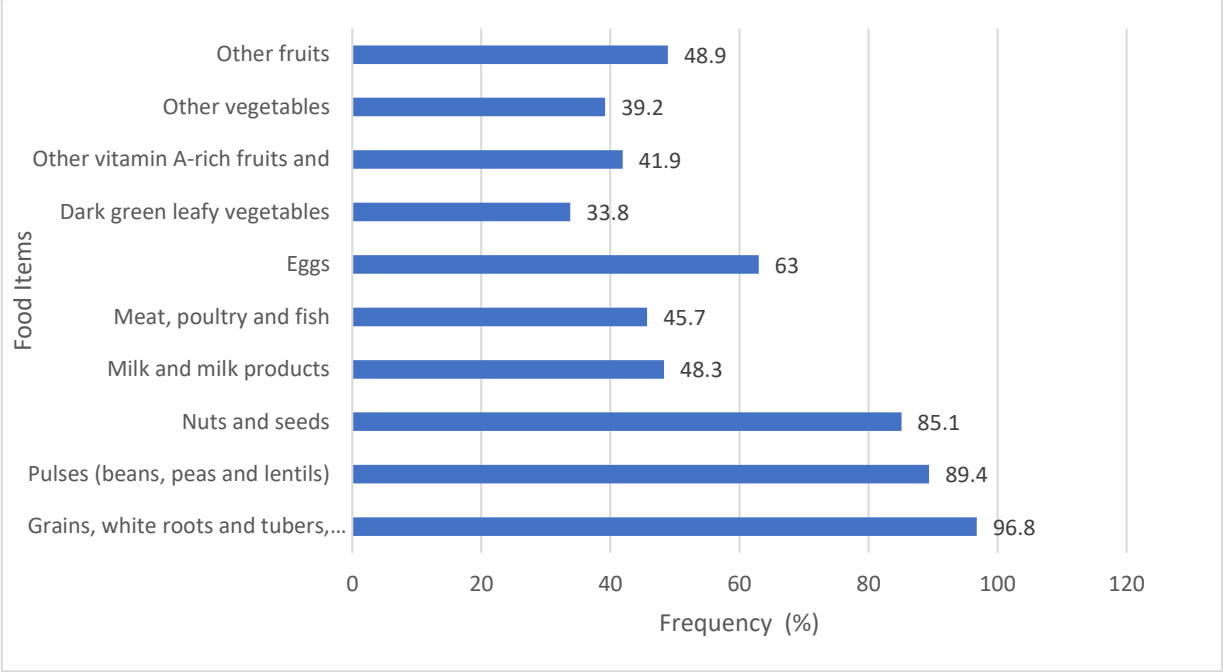


Figure 4: Dietary diversity scores, based on 24-hour recall, of pregnant women attending Antenatal care Units In public Hospitals in Addis Ababa.

Respondents with adequate dietary diversity scores were 3.4 times more likely to have adequate hemoglobin response to iron folate supplementation than respondents with inadequate dietary diversity (AOR= 3.4, 95% CI (2.12-5.56)). Also, Respondents who had inadequate intake of the proposed minimum dietary diversity were more likely to have poor hemoglobin response to iron-folate supplementation than respondents with adequate dietary diversity scores. This result is consistent with previous studies done in south Ethiopia [152], North Shewa [153] and Ghana [154] but not in line with another study conducted in Ghana [155]. The difference with the latter study done in Ghana, could be because of the inclusions of a population with better baseline nutritional status, which could mask the effects of dietary diversity on hemoglobin response.

In this study, respondents who booked for antenatal care in the first half of the first trimester and hence started IFAS earlier, had better hemoglobin response to iron folate supplementation than those who booked in the latter half of the first trimester (AOR 3.9, 95% (CI 2.41-6.56)). Similar findings have been reported in other studies conducted in Nigeria [157] and South Africa [158]. Studies have shown that iron supplementation during the period of organogenesis i.e. during the first 3- 8 weeks of gestation, could have a teratogenic effect and supplementation should not be given during this period [159]. Despite this, in a setting where anemia prevalence is high, it is recommended for pregnant women to start iron folate supplementation as early as possible after conception [10,16].

Primiparous respondents were 2.4 times more likely to have adequate hemoglobin response to iron folate supplementation than multiparous respondents (AOR= 2.4, 95% CI (1.4– 4.2)) (Table 7). The results of this study in this regard follow many reports, [160,161], but is not in line with other studies that reported a reduction in the risk of anemia with high parity [162,163]. Our finding is also consistent with a prospective follow-up study which reported that anemia and low serum ferritin levels occur more commonly in multiparous than in nulliparas [164]. This finding is also supported by a prospective cohort study conducted in Oman, Asia [165], a prospective observational study done in Japan [166], and several cross-sectional studies conducted in Pakistan [167], India [168] and Ghana [169]. Despite supplementation, progressive decline in mean Hb concentration secondary to greater acceleration of plasma volume expansion has also been reported in women with multiparity [170].

In this study, respondents who took the supplement for a duration of two months and above were 2.6 times more likely to have adequate hemoglobin response to iron folate supplementation compared to respondents who took for 1-2 months (AOR 2.6, 95% CI (1.6-4.2)) (Table 7). This finding is consistent with a study done in Kenya [117] but not in line with a study done in North Ethiopia [23]. The difference with the latter study might be due to differences in baseline nutritional status between populations which can affect the efficacy of the supplementation.

Respondents who took more than five iron folates tables per week on consecutive days, were 2.28 times more likely to have an adequate change in hemoglobin level as compared to those who took 5 or fewer tablets per week intermittently (AOR 2.28, 95% CI (1.28-4.1)). This finding is in line with a previous study [117]. In a RCT involving 200 participants, no differences was reported between alternate-day oral iron supplementation compared to daily supplementation, in increasing hemoglobin, serum ferritin or reticulocyte count [171,172]. However, the result of this study is not consistent with the previous study which assessed whether daily oral iron intake preempts anemia over intermittent intake of iron and found inconclusive results [171,173]. Also, this result contradicts with previous studies including a randomized controlled trial which found that providing iron supplements daily in divided doses increases serum hepcidin and hence reduces iron absorption [174]. This study underscored that providing iron supplements on alternate days and in single doses optimizes iron absorption and might be a preferable dosing regimen. Another study with contradictory results to our study is a randomized controlled trial which showed alternate-day dosing demonstrated a higher fractional iron absorption compared with consecutive-day dosing [175].

Participants of this study who reported that they experienced side effect/s of iron folate supplementation had poor hemoglobin response compared to those who didn't complain of side effects. Also, respondents who didn't experience side effects from IFAS were more likely to have adequate hemoglobin response to iron folate supplementation than those who experienced side effects. Pregnant women who experienced side effects from IFAS were about half as likely to have an adequate hemoglobin response compared to those who did not experience side effects from IFAS AOR=0.5 95 % CI 0.32-0.84. This might be a result of not taking the iron folate pills as per the guideline because of the side effects. Intermittent regimens of IFA tablets have been reported to have lower side effects compared with daily supplementation [103].

9. STRENGTHS AND LIMITATIONS OF THE STUDY

9.1 Strength of the study

In this study, we assessed the determinant factors and effects of iron folate supplementation in pregnant women residing in the highest capital city in the world (7,726 feet). The fact that research on the subject area being limited and the inclusion of factors not explored in previous studies, such as dietary habits and altitude, makes this study noteworthy. Additionally, as this study is conducted in a multicenter setting, this could have added value to the plausibility of the results of the study.

9.2 Limitations of the study

In this study other important diagnostic tests like serum iron level, serum ferritin level, transferrin saturation, total iron-binding capacity, reticulocyte count, RBC peripheral morphology were not performed due to cost constraints. Also, despite utilizing CBC machines from reputable manufacturers with established quality control, inherent differences between models might introduce minor variations in cell counts. Furthermore, additional blood test results for the third time were not performed due to financial problems. We were not able to follow as a cohort due to time limitations.

10. CONCLUSION & RECOMMENDATIONS

10.1 Conclusion

This study found that iron-folate supplementation improved RBC count in both anemic and non-anemic pregnant women, but it did not significantly improve leucocyte count. Moreover, platelet count decreased in both anemic and non-anemic women following supplementation. Supplementation was ineffective in preventing anemia in a substantial proportion (17%) of the women and resulted in inadequate hemoglobin response in nearly 60% of the participants. Dietary diversity, earlier timing of ANC booking, more than 2 months duration of IFAS intake, good compliance to iron folate supplementation, and primiparity were significantly associated with iron folate supplementation.

10.2 Recommendations

While iron folate supplementation remains important for preventing anemia, a more nuanced approach is needed. This study highlights the need for individualized care by considering factors like dietary habits, pregnancy history, and iron status when recommending supplementation. Emphasis has to be given for public health interventions like promoting dietary diversity, early antenatal care, and improved adherence to IFA tablets. Implementing programs for promoting dietary diversification and addressing the link between inadequate dietary intake and poor hemoglobin response is also crucial.

Emphasize should also be given to antenatal care programs iterating the importance of early and consistent antenatal care booking to ensure timely interventions for potential risk factors. Furthermore, there is a need to develop strategies to improve adherence to iron folate supplementation (IFA) tablets, considering factors like multiparity and side effects. Further research on the long-term impact of iron folate supplementation on various hematological indices, especially in women with normal iron stores is also recommended.

11. REFERENCES

[1] Kaushansky K, Lichtman MA, Prchal JT, Levi M, Burns LJ, Linch DC, editors. Williams hematology. Tenth edition. New York: McGraw-Hill; 2021.

[2] Ahenkorah B, Nsiah K, Baffoe P, Anto EO. Biochemical and hematological changes among anemic and non-anemic pregnant women attending antenatal clinic at the Bolgatanga regional hospital, Ghana. BMC Hematol 2018;18:27. https://doi.org/10.1186/s12878-018-0121-4.

[3] John E. Hall. Guyton and Hall Textbook of Medical Physiology. 14th ed. Elsevier; 2020.

[4] WHO. Global nutrition targets 2025: anaemia policy brief n.d. https://www.who.int/publications/i/item/WHO-NMH-NHD-14.4.

[5] Workineh Y, Semachew A, Ayalew E, Temesgen WA. Compliance to Iron-Folic Acid Supplementation and Its Association with the Number of ANC Visits in Ethiopia: Systematic Review and Meta-Analysis. Adv Prev Med 2019;2019:1–9. https://doi.org/10.1155/2019/3602585.

[6] Sharma D, Amgain K, Panta PP, Pokhrel B. Hemoglobin levels and anemia evaluation among pregnant women in the remote and rural high lands of mid-western Nepal: a hospital based study. BMC Pregnancy Childbirth 2020;20:182. https://doi.org/10.1186/s12884-020-02870-7.

[7] Tadesse AW, Aychiluhm SB, Mare KU. Individual and community-level determinants of Iron-Folic Acid Intake for the recommended period among pregnant women in Ethiopia: A multilevel analysis. Heliyon 2021;7:e07521. https://doi.org/10.1016/j.heliyon.2021.e07521.

[8] Tamirat KS, Kebede FB, Gonete TZ, Tessema GA, Tessema ZT. Geographical variations and determinants of iron and folic acid supplementation during pregnancy in Ethiopia: analysis of 2019 mini demographic and health survey. BMC Pregnancy Childbirth 2022;22:127. https://doi.org/10.1186/s12884-022-04461-0.

[9] Loscalzo J FA KD, Hauser S. Harrison's Principles of Internal Medicine. 21th ed. Mc Graw Hill; 2021.

[10] World Health Organization. Guideline: daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012.

[11] Cascio MJ, DeLoughery TG. Anemia. Med Clin North Am 2017;101:263–84. https://doi.org/10.1016/j.mcna.2016.09.003.

[12] Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. Am Fam Physician 2013;87:98–104.

[13] Domenica Cappellini M, Motta I. Anemia in Clinical Practice—Definition and Classification: Does Hemoglobin Change With Aging? Semin Hematol 2015;52:261–9. https://doi.org/10.1053/j.seminhematol.2015.07.006.

[14] Georgieff MK, Krebs NF, Cusick SE. The Benefits and Risks of Iron Supplementation in Pregnancy and Childhood. Annu Rev Nutr 2019;39:121–46. https://doi.org/10.1146/annurev-nutr-082018-124213.

[15] Bailey RL, West Jr. KP, Black RE. The Epidemiology of Global Micronutrient Deficiencies. Ann Nutr Metab 2015;66:22–33. https://doi.org/10.1159/000371618.

[16] Minstry Of Health-Ethiopia. National Antenatal Care Guidline. FMOH; 2022.

[17] Micronutrient Initiative, editor. Investing in the future: a united call to action on vitamin and mineral deficiencies: global report, 2009. [New Delhi: Micronutrient Initiative; 2009.

[18] Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW, et al. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. BMJ 2013;346:f3443–f3443. https://doi.org/10.1136/bmj.f3443.

[19] Dewey KG, Oaks BM. U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation. Am J Clin Nutr 2017;106:1694S-1702S. https://doi.org/10.3945/ajcn.117.156075.

[20] Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. The Lancet 2011;378:2123–35. https://doi.org/10.1016/S0140-6736(10)62304-5.

[21] Karami M, Chaleshgar M, Salari N, Akbari H, Mohammadi M. Global Prevalence of Anemia in Pregnant Women: A Comprehensive Systematic Review and Meta-Analysis. Matern Child Health J 2022;26:1473–87. https://doi.org/10.1007/s10995-022-03450-1.

[22] Woldegebriel AG, Gebregziabiher Gebrehiwot G, Aregay Desta A, Fenta Ajemu K, Berhe AA, Woldearegay TW, et al. Determinants of Anemia in Pregnancy: Findings from the Ethiopian Health and Demographic Survey. Anemia 2020;2020:1–9. https://doi.org/10.1155/2020/2902498.

[23] Belay E, Endrias A, Alem B, Endris K. Hematological responses to iron-folate supplementation and its determinants in pregnant women attending antenatal cares in Mekelle City, Ethiopia. PLOS ONE 2018;13:e0204791. https://doi.org/10.1371/journal.pone.0204791.

[24] Gebre A. Assessment of Factors Associated with Adherence to Iron-Folic Acid Supplementation Among Urban and Rural Pregnant Women in North Western Zone of Tigray, Ethiopia: Comparative Study. Int J Nutr Food Sci 2015;4:161. https://doi.org/10.11648/j.ijnfs.20150402.16.

[25] Kassa GM, Muche AA, Berhe AK, Fekadu GA. Prevalence and determinants of anemia among pregnant women in Ethiopia; a systematic review and meta-analysis. BMC Hematol 2017;17:17. https://doi.org/10.1186/s12878-017-0090-z.

[26] Ekström E, Kavishe F, Habicht J, Frongillo E, Rasmussen K, Hemed L. Adherence to iron supplementation during pregnancy in Tanzania: determinants and hematologic consequences. Am J Clin Nutr 1996;64:368–74. https://doi.org/10.1093/ajcn/64.3.368.

[27] Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010;91:1461S-1467S. https://doi.org/10.3945/ajcn.2010.28674F.

[28] Moore LG, Shriver M, Bemis L, Hickler B, Wilson M, Brutsaert T, et al. Maternal Adaptation to High-altitude Pregnancy: An Experiment of Nature—A Review. Placenta 2004;25:S60–71. https://doi.org/10.1016/j.placenta.2004.01.008.

[29] Beall CM. Adaptation to High Altitude: Phenotypes and Genotypes. Annu Rev Anthropol 2014;43:251–72. https://doi.org/10.1146/annurev-anthro-102313-030000.

[30] Windsor JS, Rodway GW. Heights and haematology: the story of haemoglobin at altitude. Postgrad Med J 2007;83:148–51. https://doi.org/10.1136/pgmj.2006.049734.

[31] Kametas NA, Krampl E, McAuliffe F, Rampling MW, Nicolaides KH. Pregnancy at high altitude: a hyperviscosity state. Acta Obstet Gynecol Scand 2004;83:627–33. https://doi.org/10.1111/j.0001-6349.2004.00434.x.

[32] Desta M, Kassie B, Chanie H, Mulugeta H, Yirga T, Temesgen H, et al. Adherence of iron and folic acid supplementation and determinants among pregnant women in Ethiopia: a systematic review and meta-analysis. Reprod Health 2019;16:182. https://doi.org/10.1186/s12978-019-0848-9.

[33] Peña-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron supplementation during pregnancy. In: The Cochrane Collaboration, editor. Cochrane Database Syst. Rev., Chichester, UK: John Wiley & Sons, Ltd; 2012, p. CD004736.pub4. https://doi.org/10.1002/14651858.CD004736.pub4.

[34] Gernand AD, Schulze KJ, Stewart CP, West KP, Christian P. Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. Nat Rev Endocrinol 2016;12:274–89. https://doi.org/10.1038/nrendo.2016.37.

[35] Mousa A, Naqash A, Lim S. Macronutrient and Micronutrient Intake during Pregnancy: An Overview of Recent Evidence. Nutrients 2019;11:443. https://doi.org/10.3390/nu11020443.

[36] Hoffman R. Hematology: basic principles and practice. 6th ed. Philadelphia, PA: Saunders/Elsevier; 2013.

[37] Conway D, Henderson MA. Iron metabolism. Anaesth Intensive Care Med 2019;20:175–7. https://doi.org/10.1016/j.mpaic.2019.01.003.

[38] Ems T, St Lucia K, Huecker MR. Biochemistry, Iron Absorption. StatPearls, Treasure Island (FL): StatPearls Publishing; 2024.

[39] Gulec S, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. Am J Physiol-Gastrointest Liver Physiol 2014;307:G397–409. https://doi.org/10.1152/ajpgi.00348.2013.

[40] Ginzburg YZ. Hepcidin-ferroportin axis in health and disease. Vitam. Horm., vol. 110, Elsevier; 2019, p. 17–45. https://doi.org/10.1016/bs.vh.2019.01.002.

[41] Georgieff MK. Iron deficiency in pregnancy. Am J Obstet Gynecol 2020;223:516–24. https://doi.org/10.1016/j.ajog.2020.03.006.

[42] Fisher AL, Nemeth E. Iron homeostasis during pregnancy. Am J Clin Nutr 2017;106:1567S-1574S. https://doi.org/10.3945/ajcn.117.155812.

[43] Wilson MJ, Harlaar JJ, Jeekel J, Schipperus M, Zwaginga JJ. Iron therapy as treatment of anemia: A potentially detrimental and hazardous strategy in colorectal cancer patients. Med Hypotheses 2018;110:110–3. https://doi.org/10.1016/j.mehy.2017.12.011.

[44] Understanding pathophysiology. Seventh edition. Place of Publication Not Identified: Mosby; 2019.

[45] James AH. Iron Deficiency Anemia in Pregnancy. Obstet Gynecol 2021;138:663–74. https://doi.org/10.1097/AOG.0000000000004559.

[46] Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000;72:257S-264S. https://doi.org/10.1093/ajcn/72.1.257S.

[47] Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016;27:89–94. https://doi.org/10.5830/CVJA-2016-021.

[48] Vinturache A, Khalil A. Fetal development and maternal adaptation. Glob libr women's me 2021. https://doi.org/10.3843/GLOWM.411323.

[49] Cunningham FG, editor. Williams obstetrics. Twenty-sixth edition. New York: McGraw Hill; 2022.

[50] Gangakhedkar GR. Physiological Changes in Pregnancy. Indian J Crit Care Med 2022;25:S189–92. https://doi.org/10.5005/jp-journals-10071-24039.

[51] Cao C, O'Brien KO. Pregnancy and iron homeostasis: an update. Nutr Rev 2013;71:35–51. https://doi.org/10.1111/j.1753-4887.2012.00550.x.

[52] Goonewardene M, Shehata M, Hamad A. Anaemia in pregnancy. Best Pract Res Clin Obstet Gynaecol 2012;26:3–24. https://doi.org/10.1016/j.bpobgyn.2011.10.010.

[53] Auerbach M. Commentary: Iron deficiency of pregnancy - a new approach involving intravenous iron. Reprod Health 2018;15:96. https://doi.org/10.1186/s12978-018-0536-1.

[54] Palgi A, Levi S, Reshef A. Anemia of pregnancy: evaluation of the effectiveness of routine dietary supplementation program in an Israeli community. Am J Public Health 1981;71:736–9. https://doi.org/10.2105/AJPH.71.7.736.

[55] Balcerek B, Steinach M, Lichti J, Maggioni MA, Becker PN, Labes R, et al. A broad diversity in oxygen affinity to haemoglobin. Sci Rep 2020;10:16920. https://doi.org/10.1038/s41598-020-73560-9.

[56] Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological Changes in Hematological Parameters During Pregnancy. Indian J Hematol Blood Transfus 2012;28:144–6. https://doi.org/10.1007/s12288-012-0175-6.

[57] Dockree S, Shine B, Pavord S, Impey L, Vatish M. White blood cells in pregnancy: reference intervals for before and after delivery. eBioMedicine 2021;74:103715. https://doi.org/10.1016/j.ebiom.2021.103715.

[58] Wang W, Sung N, Gilman-Sachs A, Kwak-Kim J. T Helper (Th) Cell Profiles in Pregnancy and Recurrent Pregnancy Losses: Th1/Th2/Th9/Th17/Th22/Tfh Cells. Front Immunol 2020;11:2025. https://doi.org/10.3389/fimmu.2020.02025.

[59] Ciobanu AM, Colibaba S, Cimpoca B, Peltecu G, Panaitescu AM. Thrombocytopenia in Pregnancy. Maedica 2016;11:55–60.

[60] Moser, Guettler, Forstner, Gauster. Maternal Platelets of the Human Placenta: Friend or Foe? Int J Mol Sci 2019;20:5639. https://doi.org/10.3390/ijms20225639.

[61] Hellgren M. Hemostasis during Normal Pregnancy and Puerperium. Semin Thromb Hemost 2003;29:125–30. https://doi.org/10.1055/s-2003-38897.

[62] James AH. Pregnancy-associated thrombosis. Hematology 2009;2009:277–85. https://doi.org/10.1182/asheducation-2009.1.277.

[63] Yoon H-J. Coagulation abnormalities and bleeding in pregnancy: an anesthesiologist's perspective. Anesth Pain Med 2019;14:371–9. https://doi.org/10.17085/apm.2019.14.4.371.

[64] Devis P, Knuttinen MG. Deep venous thrombosis in pregnancy: incidence, pathogenesis and endovascular management. Cardiovasc Diagn Ther 2017;7:S309–19. https://doi.org/10.21037/cdt.2017.10.08.

[65] Varrias D, Spanos M, Kokkinidis DG, Zoumpourlis P, Kalaitzopoulos DR. Venous Thromboembolism in Pregnancy: Challenges and Solutions. Vasc Health Risk Manag 2023;Volume 19:469–84. https://doi.org/10.2147/VHRM.S404537.

[66] Mansory EM, Alphonsus L, Hutson JR, De Vrijer B, Lazo-Langner A. Anticoagulant prophylaxis in pregnant women with a history of venous thromboembolism: A systematic review and meta-analysis. Thromb Update 2023;13:100150. https://doi.org/10.1016/j.tru.2023.100150.

[67] Sappani M, Mani T, Asirvatham ES, Joy M, Babu M, Jeyaseelan L. Trends in prevalence and determinants of severe and moderate anaemia among women of reproductive age during the last 15 years in India. PLOS ONE 2023;18:e0286464. https://doi.org/10.1371/journal.pone.0286464.

[68] WHO Global Anaemia estimates. World Health Organization; 2021.

[69] Zeisler H, Dietrich W, Heinzl F, Klaritsch P, Humpel V, Moertl M, et al. Prevalence of iron deficiency in pregnant women: A prospective cross-sectional Austrian study. Food Sci Nutr 2021;9:6559–65. https://doi.org/10.1002/fsn3.2588.

[70] Eskeland B, Malterud K, Ulvik RJ, Hunskaar S. Iron supplementation in pregnancy: is less enough?: A randomized, placebo controlled trial of low dose iron supplementation with and without heme iron. Acta Obstet Gynecol Scand 1997;76:822–8. https://doi.org/10.3109/00016349709024359.

[71] Zavaleta N, Caulfield LE, Garcia T. Changes in iron status during pregnancy in Peruvian women receiving prenatal iron and folic acid supplements with or without zinc. Am J Clin Nutr 2000;71:956–61. https://doi.org/10.1093/ajcn/71.4.956.

[72] Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997;66:1178–82. https://doi.org/10.1093/ajcn/66.5.1178.

[73] Schoorl M, Schoorl M, Van Der Gaag D, Bartels P. Effects of Iron Supplementation on Red Blood Cell Hemoglobin Content in Pregnancy. Hematol Rep 2012;4:e24. https://doi.org/10.4081/hr.2012.e24.

[74] Karakoc G, Orgul G, Sahin D, Yucel A. Is every other day iron supplementation effective for the treatment of the iron deficiency anemia in pregnancy? J Matern Fetal Neonatal Med 2022;35:832–6. https://doi.org/10.1080/14767058.2021.1910666.

[75] Seu MMV, Mose JC, Panigoro R, Sahiratmadja E. Anemia Prevalence after Iron Supplementation among Pregnant Women in Midwifes Practice of Primary Health Care Facilities in Eastern Indonesia. Anemia 2019;2019:1–8. https://doi.org/10.1155/2019/1413906.

[76] Zhao G, Xu G, Zhou M, Jiang Y, Richards B, Clark KM, et al. Prenatal Iron Supplementation Reduces Maternal Anemia, Iron Deficiency, and Iron Deficiency Anemia in a Randomized

Clinical Trial in Rural China, but Iron Deficiency Remains Widespread in Mothers and Neonates. J Nutr 2015;145:1916–23. https://doi.org/10.3945/jn.114.208678.

[77] Nh Nik R, S Mohd N. The Rate and Risk Factors for Anemia among Pregnant Mothers in Jerteh Terengganu, Malaysia. J Community Med Health Educ 2012. https://doi.org/10.4172/2161-0711.1000150.

[78] Noora Pradha SRT, Shailendra Bir Karmacharya. Effect of Anemia in Pregnancy and its Perinatal Outcome: A Prospective Cohort Study. Journal of Lumbini Medical College 2021;9:6. https://doi.org/10.22502/jlmc.v9i2.445.

[79] Seck BC, Jackson RT. Determinants of compliance with iron supplementation among pregnant women in Senegal. Public Health Nutr 2008;11:596–605. https://doi.org/10.1017/S1368980007000924.

[80] Agbozo F, Abubakari A, Der J, Jahn A. Maternal Dietary Intakes, Red Blood Cell Indices and Risk for Anemia in the First, Second and Third Trimesters of Pregnancy and at Predelivery. Nutrients 2020;12:777. https://doi.org/10.3390/nu12030777.

[81] Geta TG, Gebremedhin S, Omigbodun AO. Prevalence and predictors of anemia among pregnant women in Ethiopia: Systematic review and meta-analysis. PLOS ONE 2022;17:e0267005. https://doi.org/10.1371/journal.pone.0267005.

[82] Gebrerufael GG, Hagos BT. Anemia Prevalence and Risk Factors in Two of Ethiopia's Most Anemic Regions among Women: A Cross-Sectional Study. Adv Hematol 2023;2023:1–9. https://doi.org/10.1155/2023/2900483.

[83] Kare AP, Gujo AB. Anemia among Pregnant Women Attending Ante Natal Care Clinic in Adare General Hospital, Southern Ethiopia: Prevalence and Associated Factors. Health Serv Insights 2021;14:117863292110363. https://doi.org/10.1177/11786329211036303.

[84] Pathirathna ML, Wimalasiri KMS, Sekijima K, Sadakata M. Maternal Compliance to Recommended Iron and Folic Acid Supplementation in Pregnancy, Sri Lanka: A Hospital-Based Cross-Sectional Study. Nutrients 2020;12:3266. https://doi.org/10.3390/nu12113266.

[85] FMoH-E, EPHI. Mini Demographic Health Survey. Ministry of Health - Ethiopia; 2019.

[86] Feleke BE, Feleke TE. The Effect of Pregnancy in the Hemoglobin Concentration of Pregnant Women: A Longitudinal Study. J Pregnancy 2020;2020:1–6. https://doi.org/10.1155/2020/2789536.

[87] Brissot E, Troadec M, Loréal O, Brissot P. Iron and platelets: A subtle, under-recognized relationship. Am J Hematol 2021;96:1008–16. https://doi.org/10.1002/ajh.26189.

[88] Kadikoylu G, Yavasoglu I, Bolaman Z, Senturk T. Platelet parameters in women with iron deficiency anemia. J Natl Med Assoc 2006;98:398–402.

[89] Li X, Li N, Zhao G, Wang X. Effect of iron supplementation on platelet count in adult patients with iron deficiency anemia. Platelets 2022;33:1214–9. https://doi.org/10.1080/09537104.2022.2091772.

[90] Kurt H, Demirkiran D. The effect of iron deficiency anaemia treatment on mean platelet volume. Ir J Med Sci 1971 - 2023;192:1763–7. https://doi.org/10.1007/s11845-022-03221-5.

[91] Evstatiev R, Bukaty A, Jimenez K, Kulnigg-Dabsch S, Surman L, Schmid W, et al. Iron deficiency alters megakaryopoiesis and platelet phenotype independent of thrombopoietin. Am J Hematol 2014;89:524–9. https://doi.org/10.1002/ajh.23682.

[92] Dan K. Thrombocytosis in Iron Deficiency Anemia. Intern Med 2005;44:1025–6. https://doi.org/10.2169/internalmedicine.44.1025.

[93] Liao R, Zhou X, Ma D, Tang J, Zhong H. Iron Deficiency is Associated With Platelet Count Elevation in Patients With Dialysis-dependent Chronic Kidney Disease. J Ren Nutr 2022;32:587–94. https://doi.org/10.1053/j.jrn.2021.09.004.

[94] Keung Y-K, Owen J. Iron Deficiency and Thrombosis: Literature Review. Clin Appl Thromb 2004;10:387–91. https://doi.org/10.1177/107602960401000412.

[95] Kaushansky K. Historical review: megakaryopoiesis and thrombopoiesis. Blood 2008;111:981–6. https://doi.org/10.1182/blood-2007-05-088500.

[96] Choi SI, Simone JV. Platelet Production in Experimental Iron Deficiency Anemia. Blood 1973;42:219–28. https://doi.org/10.1182/blood.V42.2.219.219.

[97] Choi SI, Simone JV, Jackson CW. PLATELET AND MEGAKARYOCYTE KINETICS IN IRON DEFICIENCY ANEMIA. Nutr Rev 2009;32:276–9. https://doi.org/10.1111/j.1753-4887.1974.tb00974.x.

[98] Uzel V, SavaŞ S, Soker M. The cause of very severe trombocytosis: iron deficiency anemia. Hematol Transfus Cell Ther 2020;42:70. https://doi.org/10.1016/j.htct.2020.09.125.

[99] Garzon S, Cacciato PM, Certelli C, Salvaggio C, Magliarditi M, Rizzo G. Iron Deficiency Anemia in Pregnancy: Novel Approaches for an Old Problem. Oman Med J 2020;35:e166–e166. https://doi.org/10.5001/omj.2020.108.

[100] Shi H, Chen L, Wang Y, Sun M, Guo Y, Ma S, et al. Severity of Anemia During Pregnancy and Adverse Maternal and Fetal Outcomes. JAMA Netw Open 2022;5:e2147046. https://doi.org/10.1001/jamanetworkopen.2021.47046.

[101] Shi G, Zhang Z, Ma L, Zhang B, Dang S, Yan H. Association between maternal iron supplementation and newborn birth weight: a quantile regression analysis. Ital J Pediatr 2021;47:133. https://doi.org/10.1186/s13052-021-01084-7.

[102] Akalewold M, Yohannes GW, Abdo ZA, Hailu Y, Negesse A. Magnitude of infertility and associated factors among women attending selected public hospitals in Addis Ababa, Ethiopia: a cross-sectional study. BMC Womens Health 2022;22:11. https://doi.org/10.1186/s12905-022-01601-8.

[103] Peña-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. Cochrane Database Syst Rev 2015;2015. https://doi.org/10.1002/14651858.CD004736.pub5.

[104] Zofkie AC, Garner WH, Schell RC, Ragsdale AS, McIntire DD, Roberts SW, et al. An evidence-based definition of anemia for singleton, uncomplicated pregnancies. PLOS ONE 2022;17:e0262436. https://doi.org/10.1371/journal.pone.0262436.

[105] World Health Organization. Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. WHO; 2001.

[106] Okam MM, Koch TA, Tran M-H. Iron Supplementation, Response in Iron-Deficiency Anemia: Analysis of Five Trials. Am J Med 2017;130:991.e1-991.e8. https://doi.org/10.1016/j.amjmed.2017.03.045.

[107] World Bank. Poverty & Equity Brief: Africa Eastern & Southern Ethiopia [Internet] 2021. http://databank.worldbank.org/data/download/poverty/987B9C90-CB9F-4D93-AE8C-750588BF00QA/AM2020/Global_POVEQ_ETH.pdf.

[108] Anchang-Kimbi JK, Nkweti VN, Ntonifor HN, Apinjoh TO, Chi HF, Tata RB, et al. Profile of red blood cell morphologies and causes of anaemia among pregnant women at first clinic visit in the mount Cameroon area: a prospective cross sectional study. BMC Res Notes 2017;10:645. https://doi.org/10.1186/s13104-017-2961-6.

[109] Paliogiannis P, Zinellu A, Mangoni AA, Capobianco G, Dessole S, Cherchi PL, et al. Red blood cell distribution width in pregnancy: a systematic review. Biochem Medica 2018;28:030502. https://doi.org/10.11613/BM.2018.030502.

[110] Tilahun AG, Fufa DA, Taddesse RD. Undernutrition and its associated factors among pregnant women at the public hospitals of Bench-Sheko and Kaffa zone, southwest Ethiopia. Heliyon 2022;8:e09380. https://doi.org/10.1016/j.heliyon.2022.e09380.

[111] Fakier A, Petro G, Fawcus S. Mid-upper arm circumference: A surrogate for body mass index in pregnant women. S Afr Med J 2017;107:606. https://doi.org/10.7196/SAMJ.2017.v107i7.12255.

[112] Asres AW, Samuel S, Daga WB, Tena A, Alemu A, Workie SB, et al. Association between iron-folic acid supplementation and pregnancy-induced hypertension among pregnant women

in public hospitals, Wolaita Sodo, Ethiopia 2021: a case- control study. BMC Public Health 2023;23:843. https://doi.org/10.1186/s12889-023-15794-6.

[113] Arega Sadore A, Abebe Gebretsadik L, Aman Hussen M. Compliance with Iron-Folate Supplement and Associated Factors among Antenatal Care Attendant Mothers in Misha District, South Ethiopia: Community Based Cross-Sectional Study. J Environ Public Health 2015;2015:1–7. https://doi.org/10.1155/2015/781973.

[114] Minimum dietary diversity for women. FAO; 2021. https://doi.org/10.4060/cb3434en.

[115] Tahaineh L, Ayoub NM, Khassawneh AH. Evaluation of factors in a primary care setting which may cause failure to respond to oral iron treatment in iron deficiency anaemia patients. J Pharm Health Serv Res 2017;8:45–50. https://doi.org/10.1111/jphs.12149.

[116] Rajendran S, Bobby Z, Habeebullah S, Elizabeth Jacob S. Differences in the response to iron supplementation on oxidative stress, inflammation, and hematological parameters in nonanemic and anemic pregnant women. J Matern Fetal Neonatal Med 2022;35:465–71. https://doi.org/10.1080/14767058.2020.1722996.

[117] Dennis K, Marera D, Were T. Determination of hematological response to iron and folic acid supplementation among the expectant mothers attending Kakamega County Referral Hospital, Kenya. Egypt J Haematol 2022;47:262. https://doi.org/10.4103/ejh.ejh_10_22.

[118] Hoffmann JJML, Urrechaga E. Role of RDW in mathematical formulas aiding the differential diagnosis of microcytic anemia. Scand J Clin Lab Invest 2020;80:464–9. https://doi.org/10.1080/00365513.2020.1774800.

[119] Fava C, Cattazzo F, Hu Z-D, Lippi G, Montagnana M. The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype? Ann Transl Med 2019;7:581–581. https://doi.org/10.21037/atm.2019.09.58.

[120] Faghir-Ganji M, Amanollahi A, Nikbina M, Ansari-Moghaddam A, Abdolmohammadi N. Prevalence and risk factors of anemia in first, second and third trimesters of pregnancy in Iran: A systematic review and meta-analysis. Heliyon 2023;9:e14197. https://doi.org/10.1016/j.heliyon.2023.e14197.

[121] Gebreweld A, Tsegaye A. Prevalence and Factors Associated with Anemia among Pregnant Women Attending Antenatal Clinic at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Adv Hematol 2018;2018:3942301. https://doi.org/10.1155/2018/3942301.

[122] Eloísa Urrechaga LB, Jesús F Escanero. Biomarkers of hypochromia: the contemporary assessment of iron status and erythropoiesis n.d.

[123] 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;2014:1–9. https://doi.org/10.1155/2014/108593.

[124] Yilmaz G, Shaikh H. Normochromic Normocytic Anemia. StatPearls, Treasure Island (FL): StatPearls Publishing; 2024.

[125] Kumar A, Sharma E, Marley A, Samaan MA, Brookes MJ. Iron deficiency anaemia: pathophysiology, assessment, practical management. BMJ Open Gastroenterol 2022;9:e000759. https://doi.org/10.1136/bmjgast-2021-000759.

[126] Chaudhry HS KM. Microcytic Hypochromic Anemia. StatPearls Internet. Updated 2023 Aug 14], Treasure Island (FL): StatPearls Publishing; 2024.

[127] Nugraheni AI, Wijayanti KRD, Manuaba IAIM. Severe Megaloblastic Anemia & Thrombocytopenia in Pregnancy: A Case Report. Eur J Med Health Sci 2023;5:14–9. https://doi.org/10.24018/ejmed.2023.5.2.1667.

[128] Korom VG, Lueff S, Liposits A, Kellner A, Pavlovics A, Egyed M. Is iron deficiency anemia always microcytic? Pol Arch Intern Med 2020. https://doi.org/10.20452/pamw.15714.

[129] Jolobe OMP. Prevalence of hypochromia (without microcytosis) vs microcytosis (without hypochromia) in iron deficiency: Microcytosis (without hypochromia) in iron deficiency. Clin Lab Haematol 2000;22:79–80. https://doi.org/10.1046/j.1365-2257.2000.00293.x.

[130] Egyed M. Novel algorithm of anemia. Orv Hetil 2014;155:376–82. https://doi.org/10.1556/OH.2014.29806.

[131] Lin L, Ren J, Zeng C. [Mean corpuscular volume and red blood cell volume distribution width in the diagnosis of iron deficiency anemia in pregnancy]. Zhonghua Fu Chan Ke Za Zhi 1997;32:81–3.

[132] Rivera AKB, Latorre AAE, Nakamura K, Seino K. Using complete blood count parameters in the diagnosis of iron deficiency and iron deficiency anemia in Filipino women. J Rural Med 2023;18:79–86. https://doi.org/10.2185/jrm.2022-047.

[133] Sharma D. Significance of Red Cell Distribution Width in the Diagnosis of Iron Deficiency Anemia: An Observational Study from India. J Pediatr Neonatal Care 2015;3. https://doi.org/10.15406/jpnc.2015.02.00102.

[134] G S Sultana, S A Haque, T Sultana, A N Ahmed. Value of Red Cell Distribution Width (RDW) and RBC Indices in the Detection of Iron Deficiency Anemia. Mymensingh Med J 2013;22:370–6.

[135] Sultana G, Haque S, Sultana T, Rahman Q, Ahmed A. Role of red cell distribution width (RDW) in the detection of iron deficiency anaemia in pregnancy within the first 20 weeks of gestation. Bangladesh Med Res Counc Bull 2011;37:102–5. https://doi.org/10.3329/bmrcb.v37i3.9122.

[136] Viswanath D, Hegde R, Murthy V, Nagashree S, Shah R. Red Cell Distribution Width in the Diagnosis of Iron Deficiency Anemia. Indian J Pediatr 2001;68:1117–9. https://doi.org/10.1007/BF02722922.

[137] Aslan D, Gümrük F, Gürgey A, Altay Ç. Importance of RDW value in differential diagnosis of hypochrome anemias. Am J Hematol 2002;69:31–3. https://doi.org/10.1002/ajh.10011.

[138] Muckenthaler MU, Mairbäurl H, Gassmann M. Iron metabolism in high-altitude residents. J Appl Physiol 2020;129:920–5. https://doi.org/10.1152/japplphysiol.00019.2020.

[139] Department of Human Nutrition and Dietetics, Poznań University of Life Sciences, Poland, Department of Animal Physiology and Biochemistry, Poznań University of Life Sciences, Poland, Radziejewska A, Suliburska J, Kołodziejski P, Zuk E, et al. The effects of folate and iron deficiency followed by supplementation on blood morphology and inflammation biomarkers in rats. Acta Sci Pol Technol Aliment 2021;20:213–22. https://doi.org/10.17306/J.AFS.0921.

[140] Hernáez Á, Lassale C, Castro-Barquero S, Babio N, Ros E, Castañer O, et al. Mediterranean Diet and White Blood Cell Count—A Randomized Controlled Trial. Foods 2021;10:1268. https://doi.org/10.3390/foods10061268.

[141] Yu JC, Shliakhtsitsava K, Wang YM, Paul M, Farnaes L, Wong V, et al. Hematologic Manifestations of Nutritional Deficiencies: Early Recognition is Essential to Prevent Serious Complications. J Pediatr Hematol Oncol 2019;41:e182–5. https://doi.org/10.1097/MPH.0000000000001338.

[142] Park YH. Diagnosis and management of thrombocytopenia in pregnancy. Blood Res 2022;57:S79–85. https://doi.org/10.5045/br.2022.2022068.

[143] Asrie F, Enawgaw B, Getaneh Z. Prevalence of thrombocytopenia among pregnant women attending antenatal care service at Gondar University Teaching Hospital in 2014, northwest Ethiopia. J Blood Med 2017;Volume 8:61–6. https://doi.org/10.2147/JBM.S136152.

[144] Tirago D, Yemane T, Tadasa E. Magnitude of Thrombocytopenia and Associated Factors among Pregnant Women Attending the Antenatal Care Service Unit of Wachemo University Nigist Ellen Mohammed Comprehensive Specialized Hospital Hosanna, Southern Ethiopia. Adv Hematol 2024;2024:8163447. https://doi.org/10.1155/2024/8163447.

[145] Shitie D, Zewde T, Molla Y. Anemia and other hematological profiles of pregnant women attending antenatal care in Debre Berhan Referral Hospital, North Shoa, Ethiopia. BMC Res Notes 2018;11:704. https://doi.org/10.1186/s13104-018-3805-8.

[146] Taeubert MJ, Wiertsema CJ, Vermeulen MJ, Quezada-Pinedo HG, Reiss IK, Muckenthaler MU, et al. Maternal Iron Status in Early Pregnancy and Blood Pressure Throughout Pregnancy, Placental Hemodynamics, and the Risk of Gestational Hypertensive Disorders. J Nutr 2022;152:525–34. https://doi.org/10.1093/jn/nxab368.

[147] Jirakittidul P, Sirichotiyakul S, Ruengorn C, Techatraisak K, Wiriyasirivaj B. Effect of iron supplementation during early pregnancy on the development of gestational hypertension and pre-eclampsia. Arch Gynecol Obstet 2018;298:545–50. https://doi.org/10.1007/s00404-018-4821-6.

[148] Zafar T, Iqbal Z. IRON STATUS IN PREECLAMPSIA: ,. Prof Med J 2008;15:74–80. https://doi.org/10.29309/TPMJ/2008.15.01.2700.

[149] Chen S, Li N, Mei Z, Ye R, Li Z, Liu J, et al. Micronutrient supplementation during pregnancy and the risk of pregnancy-induced hypertension: A randomized clinical trial. Clin Nutr 2019;38:146–51. https://doi.org/10.1016/j.clnu.2018.01.029.

[150] Chen Z, Gan J, Zhang M, Du Y, Zhao H. Ferroptosis and Its Emerging Role in Pre-Eclampsia. Antioxidants 2022;11:1282. https://doi.org/10.3390/antiox11071282.

[151] Gumilar KE, Priangga B, Lu C-H, Dachlan EG, Tan M. Iron metabolism and ferroptosis: A pathway for understanding preeclampsia. Biomed Pharmacother 2023;167:115565. https://doi.org/10.1016/j.biopha.2023.115565.

[152] Delil R, Tamiru D, Zinab B. Dietary Diversity and Its Association with Anemia among Pregnant Women Attending Public Health Facilities in South Ethiopia. Ethiop J Health Sci 1970;28. https://doi.org/10.4314/ejhs.v28i5.14.

[153] Kibret KT, Chojenta C, D'Arcy E, Loxton D. The effect of dietary patterns on maternal anaemia in North Shewa, Ethiopia: A case–control study with Propensity Score Analysis. Nutr Health 2023:026010602311523. https://doi.org/10.1177/02601060231152345.

[154] Saaka M, Rauf AA. Role of dietary diversity in ensuring adequate haematological status during pregnancy. Int J Med Res Health Sci 2015;4:749. https://doi.org/10.5958/2319-5886.2015.00146.0.

[155] Saaka M, Oladele J, Larbi A, Hoeschle-Zeledon I. Dietary Diversity Is Not Associated with Haematological Status of Pregnant Women Resident in Rural Areas of Northern Ghana. J Nutr Metab 2017;2017:1–10. https://doi.org/10.1155/2017/8497892.

[156] Laake P, Benestad HB, Olsen BR, editors. Research in medical and biological sciences: from planning and preparation to grant application and publication. Amsterdam ; Boston: Elsevier/Academic Press; 2015.

[157] Izuka E, Obiora-Izuka C, Asimadu E, Enebe J, Onyeabochukwu A, Nwagha U. Effect of Late Antenatal Booking on Maternal Anemia and Fetus Birth Weight on Parturients in Enugu, Nigeria: An Analytical Cross-Sectional Study. Niger J Clin Pract 2023;26:558–65. https://doi.org/10.4103/njcp.njcp_117_22.

[158] M Hoque A, E Hoque M, Van Hal G. Progression of anaemia during antenatal period among South African pregnant women. Afr Health Sci 2022;22:81–92. https://doi.org/10.4314/ahs.v22i3.10.

[159] Weinberg ED. Can iron be teratogenic? BioMetals 2010;23:181–4. https://doi.org/10.1007/s10534-009-9285-5.

[160] Rizk DEE, Khalfan M, Ezimokhai M. Obstetric outcome in grand multipara in the United Arab Emirates. Arch Gynecol Obstet 2001;264:194–8. https://doi.org/10.1007/s004040000107.

[161] Kumari AS, Badrinath P. Extreme grandmultiparity: is it an obstetric risk factor? Eur J Obstet Gynecol Reprod Biol 2002;101:22–5. https://doi.org/10.1016/S0301-2115(01)00498-5.

[162] King PA, Duthie SJ, Ma HK. Grand multiparity: A reappraisal of the risks. Int J Gynecol Obstet 1991;36:13–6. https://doi.org/10.1016/0020-7292(91)90171-Z.

[163] Silva LJP. Grand grand multiparity. J Obstet Gynaecol 1992;12:301–3. https://doi.org/10.3109/01443619209015511.

[164] Imai K. Parity-based assessment of anemia and iron deficiency in pregnant women. Taiwan J Obstet Gynecol 2020;59:838–41. https://doi.org/10.1016/j.tjog.2020.09.010.

[165] Al-Farsi YM, Brooks DR, Werler MM, Cabral HJ, Al-Shafei MA, Wallenburg HC. Effect of high parity on occurrence of anemia in pregnancy: a cohort study. BMC Pregnancy Childbirth 2011;11:7. https://doi.org/10.1186/1471-2393-11-7.

[166] Habe S, Haruna M, Yonezawa K, Usui Y, Sasaki S, Nagamatsu T, et al. Factors Associated with Anemia and Iron Deficiency during Pregnancy: A Prospective Observational Study in Japan. Nutrients 2024;16:418. https://doi.org/10.3390/nu16030418.

[167] Ramesh BH, Praveen S Patil, Jennifer Joseph. Multigravidity a Major Risk Factor of Anaemia in Pregnancy and its Comparison in Primigravida Women in Raichur. Natl J Lab Med 2017;6. https://doi.org/10.7860/NJLM/2017/31498:2259.

[168] Shah T, Warsi J, Laghari Z. Anemia and its association with parity. Prof Med J 2020;27:968–72. https://doi.org/10.29309/TPMJ/2020.27.05.3959.

[169] Nonterah EA, Adomolga E, Yidana A, Kagura J, Agorinya I, Ayamba EY, et al. Descriptive epidemiology of anaemia among pregnant women initiating antenatal care in rural Northern Ghana. Afr J Prim Health Care Fam Med 2019;11. https://doi.org/10.4102/phcfm.v11i1.1892.

[170] Foo L, Somsiah P. Parity as a Determinant of the Hematologic Response to Hematinics Supplementation in Underprivileged Pregnant Women in Malaysia. Asia Pac J Public Health 1991;5:302–6. https://doi.org/10.1177/101053959100500408.

[171] Pasupathy E, Kandasamy R, Thomas K, Basheer A. Alternate day versus daily oral iron for treatment of iron deficiency anemia: a randomized controlled trial. Sci Rep 2023;13:1818. https://doi.org/10.1038/s41598-023-29034-9.

[172] Jongkraijakra S, Doungngern T, Sripakdee W, Lekhakula A. A randomized controlled trial of thrice-weekly versus thrice-daily oral ferrous fumarate treatment in adult patients with iron-deficiency anemia. Ann Hematol 2023;102:1333–40. https://doi.org/10.1007/s00277-023-05198-2.

[173] Uçan A, Kaya ZI, Yilmaz EÖ, Vasi İ, Özgeyik, MO. Comparing therapeutic effects of alternate day versus daily oral iron in women with iron deficiency anemia: A retrospective cohort study. Medicine (Baltimore) 2023;102:e34421. https://doi.org/10.1097/MD.0000000000034421.

[174] Stoffel NU, Cercamondi CI, Brittenham G, Zeder C, Geurts-Moespot AJ, Swinkels DW, et al. Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomised controlled trials. Lancet Haematol 2017;4:e524–33. https://doi.org/10.1016/S2352-3026(17)30182-5.

[175] Goodsall TM, Walker T. Iron absorption from oral iron supplements given on consecutive versus alternate days in iron-depleted women. BMJ Evid-Based Med 2018;23:228–9. https://doi.org/10.1136/bmjebm-2018-111013.

12. ANNEXES

ANNEX I. Information sheet and Informed Consent form (English version)

Good morning/Afternoon?

How are you? My name is ----------. I am a data collector for the study which is going to be conducted in public hospitals located in Addis Ababa. This hospital is one of the study areas in which data will be collected for the study "Determinants and effects of Iron Folic acid supplementation on maternal Hematological indices among pregnant women" by Zeleke Endalew who is studying his master's degree at Addis Ababa university College of Medicine and Health science, School of medicine physiology department.

You are kindly being asked to be participant of this study. Before you decide, it is important for you to read the following information carefully and ask if there is anything that is not clear to take part in the study. The purpose of this study is to assess the effect IFA supplementation on maternal red blood cells hemoglobin content and determinant factors. As i have mentioned above, this study is just for research purpose but we hope that the information obtained will be used to inform hospitals and other policy formulators the potential result of which could be improvement in healthcare service delivery in the country.

Title: Assessment of Determinants and effect IFA supplementation on maternal hematological indices at Addis Ababa city public hospitals, Ethiopia, 2023.

Producer and duration: The study involves drawing/taking blood sample from your veins located at the antecubital fossa, to be sent for analysis. Every data collected will be kept confidentially. Risk and benefits: There are no risk involved in this study. You may feel some pain while blood is being withdrawn but this will not have bad consequence. There are no direct medical benefits to you for participating in this study. A potential benefit of the study will be improvement of the health service delivery based on the recommendation of this study findings.

Confidentiality: This research will be conducted in accordance with all the Ethiopian laws and regulations that protect rights of human research subjects. All records and other information obtained will be kept strictly confidential and your protected health information will not be used without permission. All data collection tools will be identified by codes or number to protect any information that could be used to identify you as a participant. Results of this study may be published but no name or other identifying information will be released.

Volunteer participation: it is up to you to decide whether to take part in this study. Refusal to participate or the decision to withdraw from this research will involve no penalty or loss of benefits and this will not affect your relationship with the investigator and the treating team.

Cost and compensation to participant: There won't be compensation to the subjects for participation in this study.

Institution review Board: this study has been approved by the institutional research and ethics committee of Addis Ababa university, college of medicine and health science, school of medicine. It has got permission from this hospital too.

Contact address: If there are any questions or enquires at any time about the study or procedures, please contact the PI Using the following address:

Principal investigator: Zeleke Endalew

E-mail: zeleke.endalew@aau.edu.et , Mobile phone: +251910804000

Are you willing to participate? If the answer is yes, please continue

By signing this consent form, I confirm I have read/heard form and have had the opportunity to ask questions. I voluntarily agree to take part in this study.

Code of the participant ……signature……. date…….

ANNEX II. English version Questionnaire

Hospital Name----------Date……Time… pregnancy stage_____weeks

Subject Code_____

Table 8: English version data collection checklist form to assess determinants and effects of IFA supplementation in maternal Hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa.

Part I: Maternal Socio-Demographic Related Characteristics to assess determinants and effects of IFA supplementation in maternal Hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa.

No	Socio demographic factors	Response	Skip to
100	Age of the mother	_____ years	
101	Marital status	1. Single 2. Married 3. Divorced 4. Widowed	
102	Educational status	1. Can't read and write 2. Can read and write 3. Primary school 4. Secondary School 5. College and above	
103	Occupation	1. Governmental Employee 2. Private 3. Marchant 4. Farmer 5. House Wife 6. others specify….	
104	Residence	1. urban 2. rural	
105	Average monthly income	-------ETB	
106	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others, please specify	

Part II: Maternal Medical and Obstetric Predictors to assess determinants and effects of IFA supplementation in maternal hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa.

No	Maternal Medical and Obstetric Predictors	Response	
201	RH factor and blood group	1. --------	
202	Stage of the pregnancy	2.----------weeks	
203	Starting date of ANC follow-up	Starting from --------- week of gestation	
204	Parity/Gravidity	1. Nulliparous 2. Primiparous 3. Multipara 4. Grand-multiparous	
205	Birth interval	1. ----------years	
206	Did you have earlier diagnosis of the bellow mentioned chronic diseases?	1. Yes 2. No	If No skip to 208
207	If yes, one of the chronic medical problems (multiple response Possible)	1. DM 2. HTN 3. Anemia 4. HIV/AIDS 5. Covid 19 6. Other specify ----	
208	For how many weeks did you take the IFA tablets.	---------- weeks	
209	Did you have any of the mentioned possible side effects of the iron supplementation?	Constipation Diarrhea stomach cramps/upset stomach	
210	Did you pay to get the IFA tablets?	1. Yes 2. No	
211	From where did you get the IFA tablets?	1. Public Health care centers 2. Private Health Care facility	
212	Did you have diagnosis of anemia during your earlier pregnancy or prior to the first pregnancy (if you are primigravida)?	1.Yes 2. No	
213	Did you have PPH during your earlier delivery (where you told by your birth attendant that you had PPH? (For multigravida women)	1. Yes 2. No	

214	Did you take IFA supplement during your earlier pregnancy? If yes please specify for how long you took the tablets. And from where you took it.	1. Yes, for ____weeks 2. No	
215	Do you consume the bellow mentioned items most often, during your pregnancy? Please specify how often if your answer is yes. a. Tea b. Coffee c. Wine	1. Yes 2. No	
216	If yes, when do you often take these drinks in relation to meal time?	1. Before meal 2. After meal	
217	If you didn't take IFA supplement during your earlier pregnancy, what was the reason for not taking the tablets?	1. I was not prescribed with IFA tablets 2. Forgetfulness 3. Side effects 4. I don't think it helps 5. others	
218	Have you been fasting? If yes, since when?	1. Yes, for week/s/month/ 2. No, I did not	
219	How often do you eat in a day?	_____ times a day	
220	How many of the following listed food items did you consume in the last 24 hours? Please select all that you ate. (Circle the number/s)[DDS]	Food groups consumed (Food items within the group) **1. Grains** Teff, wheat, corn/maize, barley, rice, sorghum, millet oats, enjera, bread, porridge, kita, chechebessa, kolo, nifiro, noodles (pasta, spaghetti) **White roots and tubers, and plantains:** Potatoes (all skin colours), enset (kocho, bulla or amicho), sweet potato (white/pale yellow fleshed), white yam (boye), cassava, taro (godere) **2. Beans, peas, lentils, or chickpea** **3. Nuts or seeds** Groundnut/peanut, seeds (like sesame) and seed "butters"	

		4. Milk and milk products (Cheese, yoghurt) 5. **Meat, poultry and fish** Organ meat (Liver, kidney, heart, gizzard) Red flesh mammals' meat (Beef, lamb, goat) Poultry meat (Chicken) Fish and seafood (Fresh, frozen, dried or canned fish) **6. Eggs** 7. **Dark green leafy vegetables** (Kale, broccoli, lettuce, spinach, and Swiss chard) 8. **Vitamin A rich vegetables or roots** (Pumpkin, carrots, squash, or orange flesh sweet potatoes) Vitamin A rich fruits (Ripe mangoes or ripe papaya) **9. Other vegetables (**Tomato, eggplant, green pepper, cucumber, cabbage (common and red varieties), cauliflower, mushroom, and zucchini, beans, peas or lentils when the fresh/ green pod is consumed**)** **10. Other fruits (**Orange, banana, avocado, pineapple, guava, watermelon, apple, grapefruit, berries**)**	
221	Is there any medication that you are taking now? If yes please specify it and for what diagnosis you are taking it? Also write if you are taking/took multivitamin	Medications 1. Yes 2. No Multivitamins 1. Yes. 2. No	
222	Were you taking calcium tablets? If yes, do you take IFAS tablets with iron simultaneously?	1. Yes 2. No	
223	Have you received or donated blood in the last 2 months	1. Yes 2. No	
224	Laboratory measurements performed before IFAS initiation i.e baseline hematological analysis.	1. WBC = 2. Hb= 3. HCT= 4. RBC= 5. MCV= 6. MCH= 7. MCHC= 8. RDW = 9. Platelet Count =	

225	Laboratory measurements at endpoint of the follow-up	1. WBC = 2. Hb= 3. HCT= 4. RBC= 5. MCV= 6. MCH= 7. MCHC= 8. RDW = 9. Platelet Count =	
226	Was your pregnancy planned?	1. Yes 2. No	
227	BMI .	1. Weight ____Kg (pre-pregnancy if available 2. Height? ____m BMI= _____kg/m2 (Using Pre- pregnancy wt) 3. MUAC= _____ ____	
228	Blood pressure	1. Systolic Blood Pressure _____ mm hg 2. Diastolic Blood pressure _____ mm hg	

Table 9. Minimum dietary diversity for women of reproductive age (18_49 years), Ethiopia extensive food list[114]

	Food groups	Food items
A	**Foods made from grains**	Teff, wheat, corn/maize, barley, rice, sorghum, millet, oats, enjera, bread, porridge, kita, chechebessa, kolo, nifiro, noodles (pasta, spaghetti)
B	**White roots and tubers or plantains**	Potatoes (all skin colours), enset (kocho, bulla or amicho), sweet potato (white/pale yellow fleshed), white yam (boye), cassava, taro (godere)
C	**Beans, peas, lentils**	Beans, peas, lentils, or chickpea
D	**Nuts or seeds**	Groundnut/peanut, seeds (like sesame) and seed "butters"
E	**Milk**	Milk
F	**Milk products**	Cheese, yoghurt
G	**Organ meat**	Liver, kidney, heart, gizzard
H	**Red flesh mammals' meat**	Beef, lamb, goat
I	**Processed meat**	–
J	**Poultry and other white meats**	Chicken
K	**Fish and seafood**	Fresh, frozen, dried or canned fish
L	**Eggs**	Eggs
M	**Dark green leafy vegetables**	Kale, broccoli, lettuce, spinach, and Swiss chard
N	**Vitamin A rich vegetables or roots**	Pumpkin, carrots, squash, or orange flesh sweet potatoes
O	**Vitamin A rich fruits**	Ripe mangoes or ripe papaya
P	**Other vegetables**	Tomato, eggplant, green pepper, cucumber, cabbage (common and red varieties), cauliflower, mushroom, and zucchini, beans, peas or lentils when the fresh/ green pod is consumed

Q	**Other fruits**	Orange, banana, avocado, pineapple, guava, watermelon, apple, grapefruit, berries
R	**Packaged salty snacks**	Crisps and chips

ANNEX III. Information sheet and Informed Consent form (Amharic version)

ሰላም ጠና ይስጥልኝ፤

ስሜ ---------- እባላለሁ፤ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሁለተኛ ዲግሪያቸውን በመማር ላይ በሚገኙት ዘለቀ እንዳለው በአዲስ አበባ ውስጥ በሚገኙ የመንግስት ሆስፒታሎች ለሚካሄድ ጥናት መረጃ ሰብሳቢ ነኝ። የጥናቱ አርስት 'እርግዝና ላይ በሚገኙ አይረንና ፎሊክ አሲድ እንክብሎችን በሚዎስዱ እናቶች የሂሞግሎቢን ይዘት እና ወሳኝ ሁኔታዎች ላይ የሚያሳድረው ተፅዕኖ ይሰኛል። ለጥናት መረጃ ከሚሰበሰብበት አንዱ ይህ ሆስፒታል ነው ። የዚህ ጥናት ተሳታፊ እንድትሆኑ በአክብሮት ተጠይቀዋል። ከመወሰንዎ በፊት የሚከተለውን መረጃ በጥንቃቄ ማንበብ እና በጥናቱ ውስጥ ለመሳተፍ ግልጽ ያልሆነ ነገር ካለ ይጠይቁ። የዚህ ጥናት ዓላማ የአይረንና ፎሊክ አሲድ እንክብሎችን በሚዎስዱ እናቶች በእናቶች ቀይ የደም ሴሎች የሂሞግሎቢን ይዘት እና በሚወስኑ ምክንያቶች ላይ ያለውን ተጽእኖ ለመገምገም ነው፤ ከላይ እንደገለጽኩት ይህ ጥናት ለምርምር ዓላማ ብቻ ነው ነገር ግን የተገኘው መረጃ ለሆስፒታሎች እና ለሌሎች በሀገሪቱ ያለውን የጤና አጠባበቅ አገልግሎት አሰጣጥ መሻሻል ሊያመጣ የሚችል ውጤት ለማሳወቅ ይጠቅማል ተብሎ ይታሰባል።

የጥናቱ ሂደትና እና የቆይታ ጊዜ፡ ጥናቱ እጅዎ ውስጥ ከሚገኙት ደም ስር ናሙና መውሰድ/ መውሰድን ያካትታል። እያንዳንዱ የተሰበሰበ መረጃ በሚስጥር ይጠበቃል። በዚህ ጥናት ውስጥ በመሳተፍዎ ምንም አይነት ስጋት የለም። ደም በሚዎሰድበት ጊዜ ትንሽ ህመም ሊሰማዎት ይችላል ፤ ነገር ግን ይህ ጉዳት አይኖረውም. በዚህ ጥናት ውስጥ ለመሳተፍ ምንም አይነት ቀጥተኛ የህክምና ጥቅሞች የሉም። የጥናቱ ፋይዳ በዚህ የጥናት ግኝቶች ላይ የተመሰረተ የጤና አገልግሎት አሰጣጥ መሻሻል ነው።

ሚስጥራዊነት፡- ይህ ጥናት የሚካሄደው በሁሉም የኢትዮጵያ ህግጋትና መመሪያዎች መሰረት የሰው ልጅ የምርምር ጉዳዮችን መብቶች በሚያስከብር መልኩ ነው። ሁሉም መዛግብት እና ሌሎች የተገኙ መረጃዎች በጥብቅ ሚስጥራዊ ይሆናሉ እና የተጠበቀው የጤና መረጃዎ ያለፈቃድ ጥቅም ላይ አይውልም። እርስዎን እንደ ተሳታፊ ለመለየት የሚያገለግል ማንኛውንም መረጃ ለመጠበቅ ሁሉም የመረጃ መሰብሰቢያ መሳሪያዎች በኮዶች ወይም በቁጥር ይታወቃሉ። የዚህ ጥናት ውጤት ሊታተም ይችላል ነገር ግን ስም ወይም ሌላ መለያ መረጃ አይወጣም። በዚህ ጥናት ውስጥ ለመሳተፍ መወሰን የእርስዎ ነው። ለመሳተፍ ፈቃደኛ አለመሆን ወይም ከዚህ ጥናት ለመውጣት መወሰን ምንም አይነት ቅጣት ወይም ጥቅማጥቅሞችን ማጣት አያካትትም እና ይህ ከመርማሪው እና ከህክምና ቡድን ጋር ያለዎትን ግንኙነት አይጎዳውም።

ለተሳታፊ ወጪ እና ማካካሻ፡ በዚህ ጥናት ውስጥ ለሚሳተፉት ምንም አይነት ቀጥተኛ ጥቅም አይኖርም። የተቋማት ገምጋሚ ቦርድ፡ - ይህ ጥናት በአዲስ አበባ ዩኒቨርሲቲ፣ በሕክምናና ጤና ሳይንስ ኮሌጅ፣ በሕክምና ትምህርት ቤት ተቋማዊ ጥናትና ሥነ ምግባር ኮሚቴ ጸድቋል። ከዚህ ሆስፒታልም ፈቃድ አግኝቷል።

የተመራማሪ አድራሻ፡- ስለ ጥናቱ ወይም አካሄዶቹ በማንኛውም ጊዜ ብዥታ ወይም ጥያቄዎች ካሉ፣ እባክዎን በሚከተለው አድራሻ ተመራማሪውን ያግኙ።

ዋና ተመራማሪ ስም ፡ ዘለቀ እንዳለው

ኢሜል፡ zeleke.endalew@aau.edu.et

ሞባይል ስልክ፡ +251910804000

ለመሳተፍ ፈቃደኛ ነዎት? መልስዎ አዎ ከሆነ፣ እባክዎ ይቀጥሉ

ይህን የስምምነት ቅጽ በመፈረም ፎርሙን እንዳነበብኩ/እንደሰማሁ እና ጥያቄዎችን ለመጠየቅ እና ከላይ የተጠቀሱትን መረጃዎች ለመስጠት እንደተስማማሁ አረጋግጣለሁ። በዚህ ጥናት ለመሳተፍ በፈቃዴ ተስማምቻለሁ።

የተሳታፊው ኮድ..... ፊርማ .....ቀን .....

ANNEX IV. Amharic version Questionnaire

Table 10: Amharic version data collection checklist form to assess determinants and effects of IFA supplementation in maternal Hematological indices among pregnant women attending ANC Units in Public Hospitals in Addis Ababa.

ክፍል አንድ

ቁጥር	ሶሲዮዮ ዲሞግራፊ ስታተስ	መልስ
100	እድሜ	----------አመት
101	የጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. ተፋትቻለህ 4. ሌላ
102	የትምህርትሁኔታ	የእናት የትምህርት ሁኔታ 1. ማንበብና መጻፍ አልችልም 2. ማንበብና መጻፍ እችላለሁ 3. የመጀመሪያ ደረጃ 4. ሁለተኛ ደረጃ 5. ኮሌጅ
		የባለቤት የትምህርት ሁኔታ 1. ማንበብና መጻፍ አልችልም 2. ማንበብና መጻፍ እችላለሁ 3. የመጀመሪያ ደረጃ 4. ሁለተኛ ደረጃ 5. ኮሌጅ
103	ስራ	1. የመንግስት ሰራተኛ
		2. ነጋዴ 3. የግብርና ስራ 4. የቤት እመቤት 5. ሌላ ….
104	በቋሚነት የሚኖሩበት ቦታ	1. ገጠር 2. ከተማ
105	አማካይ ወርሃዊ የገቢ መጠን	-------ብር
106	ሃይማኖት	1. ኦርቶዶክስ ክርስቲያን 2. ሙስሊም 3. ፕሮቴስታንት 4. ካቶሊክ 5. ሌላ
107	የቤተሰብ መጠን (በቤትዎ ዉስጥ ምን ያክል ሰው ይኖራል?)	

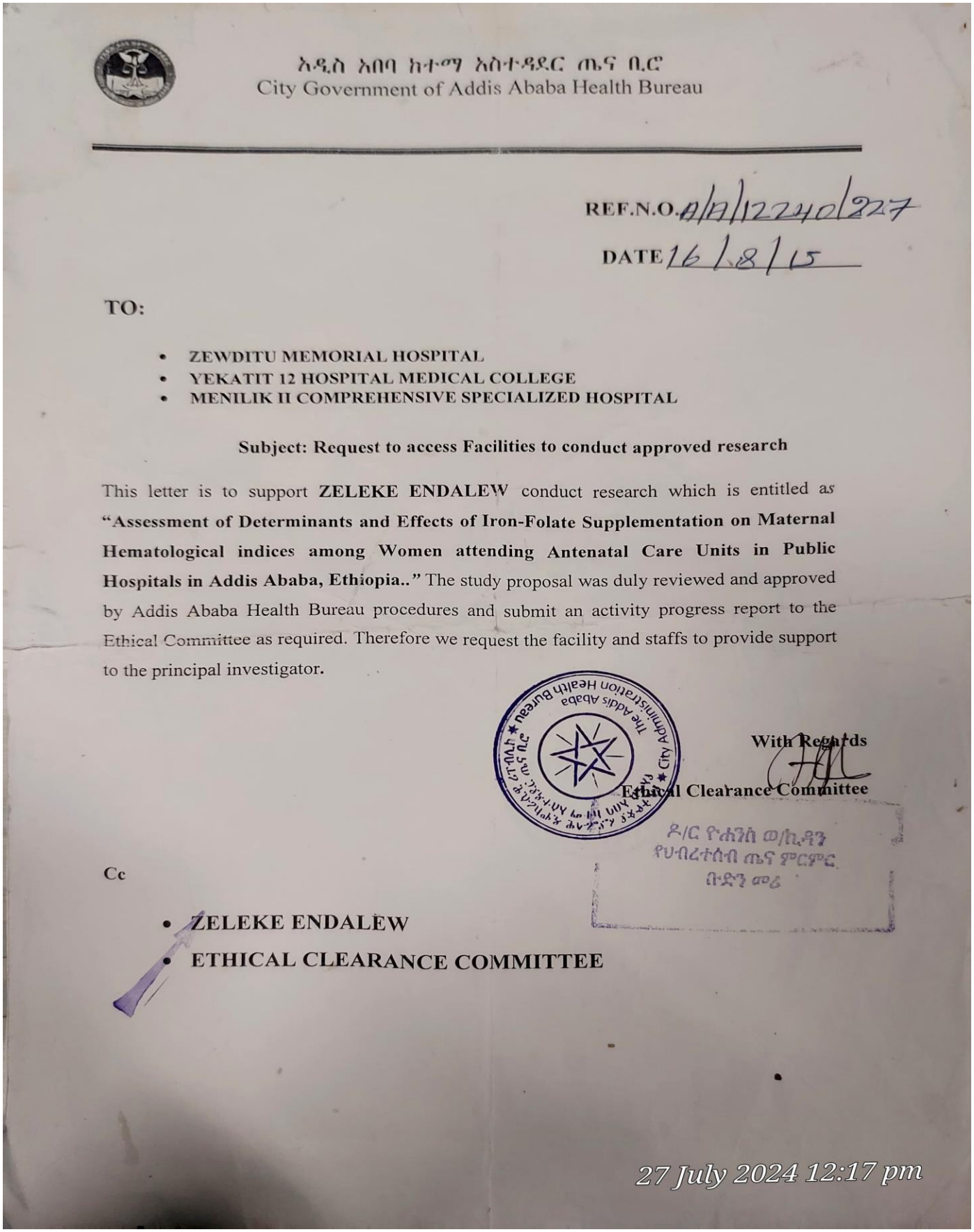
ክፍል ሁለት፡ የተሰታፊዎችን ሜዲካልና ኦብስተትሪክ ሁኔታን በተመለከተ

ቁ	ሜዲካልና ኦብስተትሪክ ፕሪዲክተርስ	መልስ
201	የደም አይነት	--------
202	የእርግዝና የእድገት ደረጃ	---------- weeks
203	የ እርግዝና ክትትል የጀመሩበት ቀን	ከ ---------የ እርግዝና ሳምንት ጀምሮ
204	ግራቪዲቲ/ፓሪቲ	1. ፕሪሚፓ/Primipara 2. መልቲፓ/Multipara 3. ፕሪሚግራቪድ/Primigravida 4. መልቲግራቪድ/Multigravida
205	የወሊድ ርቀት/በርዝ ኢንተርቫል	1---------አመት
206	ከዚህ በመቀጠል የተዘረዘሩት የጤና እክሎች ነበረቦት?	1. አ 2. ዎ አ ይ
	አዎ ከሆነ ከሚከተሉት ዉስጥ የትኛው የጤና እክል እንደነበረቦት ይግለጹ።	1. ይስኳር በሽታ 2. የደም ግፊት በሽታ 3. የደም ማነስ በሽታ 4. የኩላሊት በሽታ()ለረጅም ጊዜ የቆየ) 5. ኮቪድ 19 3. ሌሎች ----
207	የደም ማነስ እንክብሎ ለምን ያክል ጊዜ ወስደዋል	ለ---------- ሳምንት
208	የደም ማነስ እንክብሎ እየወሰዱ ሳለ ከተዘረዘሩት የጎን ዮሽ ጉዳት ተሰምቶት ያውቃል?	ı. የሆድ ድርቀት _____ 2. ተቅማጥ _____ 3. የሆድ ቁርጠት _____
209	በመጀመሪአ የእርግዝና ክትትልዎ ጊዜ የደም ማነስ እንክብሎችን ገዝተው እንዲጠቀሙ በባለሙያ ታዘው ነበር ?	1. አዎ 2. አይ
210	የደም ማነስ እንክብሎችን ከየት ነበር የወሰዱት	1. ከመንግስት የህክምና ተቋም በነጻ ____ 2. ከግል ፋርማሲ ____
211	መልስዎ ከግል ፋርማ ከሆነ ወደ ግል ግል ፋርማሲ ሊሄዱ ያቻሉት በምን ምክንያት ነበር?	1. ከመንግስት ጤና ተቋም የደም ማነስ እንክብሎች እንደሌሉ ስለተነገረኝ እኔ ብግሌ መግዛት ስለፈለኩ
212	ከዚ ቀደም በነበረ እርግዝና ጊዜ/ከ እርግዝና በፊት የደም እጥረት እንደነበረቦት በ ጤና ባለሙያ ተነግሮት ነበር?	አዎ_____ አይ_____
213	የቅርብ ጊዜ ልጅዎን ሲወልዱ ከፍተኛየሆነ የደም መፍሰስ ገጥሞት እንደነበር ባዋለዶት የጤና ባለሙያ ተነግሮት ነበር?	አዎ_____ አይ___
214	ከዚ ቀደም በነበሮት እርግዝና ጊዜ የደም ማነስ እንክብሎችን ወስደው ነበር? ወስደው ከነበር ለምን ያክል ጊዜ ነበር የወሰዱት?	አዎ፣ለ_____ሳምንት ወስጃለሁ አይ፣ አልወሰድኩም

215	በ እርግዝና ጊዜ የተጠቀሱትን የመጠጥ አይነቶች በምን ያክል ድግግ ሞሽ ይወስዳሉ (ወስደው የማያቁ ከሆነ ወስጀ አላውቅም ተብሎ ይሞላ)	1. ሻይ _____ 2. ቡና_____ 3. ወይን_____
216	ከላይ የተጠቀሱትን መጠጦች አብዛኛውን ጊዜ የሚጠቀሙት ከምግብ በፊት ወይስ በኋላ?	4. ሌላ_____ a. ከምግብበፊት _____ b. ከምግብ በኋላ_____
217	በባለፈው እርግዝናዎት ጊዜ የደም ማነስ እንክብሎችን ወስደው ካልነበረ ምክኛትዎ ምንድን ነበር?	1. ስላልታዘዘልኝ 2. በጎን ዮ ሽ ጉዳት ምክን ያት 3. ይጠቅማል ብየ ስላላሰብኩ 4. ጤና ተቋም የእርግዝናክትትል ስላልነበረኝ
218	ባአለፉት ሁለተ ወራት ዉስጥ ጾም የጾሙበት ቀን አለ ? አዎ ከሆነ፣ ከመቼ ጀምሮ?	1. አዎ፣ ለ____ሳምንት/በወር/ 2. አይ, አላደረግኩም
219	በቀን ዉስጥ በ አማካይ ስንት ጊዜ ይመገባሉ?	
220	ባለፉት 24 ሰዓታት ውስጥ ከሚከተሉት የተዘረዘሩ ምግቦች ውስጥ ምን ያህሉን ተመገቡ? እባኮትን የበሉትን ሁሉ ይምረጡ። (ቁጥሩን ይክበቡ)	1. ጥራጥሬዎች ጤፍ፣ ስንዴ፣ በቆሎ/በቆሎ፣ ገብስ፣ ሩዝ፣ ማሽላ፣ ማሽላ አጃ፣ ኤንጀራ፣ ዳቦ፣ ገንፎ፣ ኪታ፣ ጨጨጨሳ፣ ቆሎ፣ ንፍሮ፣ ኑድል (ፓስታ፣ ስፓጌቲ)
		ሌሎች ድንች (ሁሉም የቆዳ ቀለሞች)፣ እንሰት (ኮቾ፣ ቡላ ወይም አሚቾ)፣ ድንች (ነጭ/ሐመር ቢጫ ሥጋ ያለው)፣ ነጭ ያም (ቦይ)፣ ካሳቫ፣ ጣሮ (ጎደሬ)
		2. ባቄላ፣ አተር፣ ምስር ወይም ሽንብራ
		3. ለውዝ፣ ዘር (እንደ ሰሊጥ)
		4. ወተት እና የወተት ተዋጽኦዎች (አይብ፣ እርጎ) ወተት እና የወተት ተዋጽኦዎች (አይብ፣ እርጎ) 5. የሰውነት አካል ሥጋ (ጉበት ፣ ኩላሊት ፣ ልብ) ሥጋ (የበሬ ሥጋ ፣ በግ ፣ ፍየል) የዶሮ ሥጋ (ዶሮ) ዓሳ እና የባህር ምግቦች (ትኩስ ፣ የቀዘቀዘ ፣ የደረቁ ወይም የታሸጉ ዓሳ) 6. እንቁላል 7. ጥቁር አረንጓዴ ቅጠላማ አትክልቶች (ካሌ፣ ብሮኮሊ፣ ሰላጣ፣ ስፒናች እና የስዊስ ቻርድ) 8. በቫይታሚን ኤ የበለጸጉ አትክልቶች ወይም ሥሮች (ዱባ፣ ካሮት፣ ወይም ብርቱካን ሥጋ ስኳር ድንች) በቫይታሚን ኤ የበለጸጉ ፍራፍሬዎች (የበሰለ ማንጎ ወይም የበሰለ ፓፓያ) 9. ሌሎች አትክልቶች (ቲማቲም ፣ ኤግፕላንት ፣ አረንጓዴ በርበሬ ፣ ዱባ ፣ ጎመን (የተለመዱ እና ቀይ ዝርያዎች) ፣ ጎመን ፣ እንጉዳይ እና ዝኩኒ ፣ ባቄላ ፣ አተር ወይም ምስር 10. ሌሎች ፍራፍሬዎች (ብርቱካን, ሙዝ, አቮካዶ, አናናስ, ጉዋቫ, ሐብሐብ, ፖም, ወይን ፍሬ, ቤሪ
	እየወሰዱት ያሉት መድሃኒት ወይም መልቲቫይታሚን እንክብሎች አሉ? ካለ መድሃኒቲ	መድሃዲኒት፡ ----------

221	ምን እንደሆነና ለምን እንደታዘዘልዎት ይግለጹ። የ ራይት ምልክት ይጠቀሙ።	መልቲቫይታሚን ታብሌት ፡ _____
222	ካልሴም ታብለት እየወሰዱ ነው? መልስዎ አዎ ከሆነ የደም ማነስ የታዘዙልዎትን ታብሌት ከ ካልቼም ጋር አብረው ነው ሚዎስዱት? መልስዎ አይደለም ከሆነ በስንት ሰአት ልዩነት/ርቀት ነው ይወስዱ የነበር/እየወሰዱ ያለ?	_____ _____ _____
223	በቅርብ ጊዜ ደም ለግሰው ነበር ?	አዎ_____ አይ _____
224	አይረንና ፎሊክ አሲድ እንክብሎችን መውሰድ ከመጀመሩ በፊት የተደረገ የላብራቶሪ ናሙና ምርመራ ዉጤት	1. WBC = _____ 2. Hb=_____ 3. HCT=_____ 4. RBC= _____ 5. MCV= _____ 6. MCH= _____ 7. MCHC= _____ 8. RDW = _____ 9. Platelet Count = _____
225	ሌሎች ሜዝርመንቶች (ኢሮን ፎሊክ አሲድ እንክብሎችን መውሰድ ከጀመሩ ቢያንስ ከ አንድ ወር በኋላ የተደረገ ምርመራ)	1. የደም ዉስጥ የግሉኮስ መጠን _____ 2. የደም ግፊት ልኬት _____
226	አይረንና ፎሊክ አሲድ እንክብሎችን ከወሰዱ ቢያንስ ከ አንድ ወር በኋላ የተደረገ የላብራቶሪ ናሙና ምርመራ ዉጤት	1. WBC = _____ 2. Hb= _____ 3. HCT=_____ 4. RBC= _____ 5. MCV= _____ 6. MCH= _____ 7. MCHC= _____ 8. RDW = _____ 9. Platelet Count = _____
227	እርግዝናዎት የታቀደ ነበር?	1. አዎ 2. አይ
228	ፕረዘንት ፕሪናታል ሪከርድ	1. ክብደት_____ኪ/ግ (ቅድመ ወሊድ) 2. ቁመት_____ሜ ቢኤም አይ =_____ኪሎ ግራም ፐር ሜትር ስኳር (ከ እርግዝና በፊት የተደረገ የክብደት መጠንንበመጠቀም) 3. የደም ግፊት መጠን ? ሲስቶሊክ = _____ሚሊሜ ትር ሜርኩሪ ዲያስቶሊክ =__ _____ሚሊ ሜትር ሜርኩሪ 4.ሚክ (ሚድ አፐር አርምሰርከምፊረንስ)= _____

ANNEX V. Letter of Approval (Ethical Clearance Letters)



አዲስ አበባ ከተማ አስተዳደር ጤና ቢሮ
City Government of Addis Ababa Health Bureau

REF.N.O. ሰ/ክ/12240/217

DATE 16/8/15

TO:

- ZEWDITU MEMORIAL HOSPITAL
- YEKATIT 12 HOSPITAL MEDICAL COLLEGE
- MENILIK II COMPREHENSIVE SPECIALIZED HOSPITAL

Subject: Request to access Facilities to conduct approved research

This letter is to support **ZELEKE ENDALEW** conduct research which is entitled as **"Assessment of Determinants and Effects of Iron-Folate Supplementation on Maternal Hematological indices among Women attending Antenatal Care Units in Public Hospitals in Addis Ababa, Ethiopia.."** The study proposal was duly reviewed and approved by Addis Ababa Health Bureau procedures and submit an activity progress report to the Ethical Committee as required. Therefore we request the facility and staffs to provide support to the principal investigator.

The Addis Ababa City Administration Health Bureau

With Regards

Ethical Clearance Committee

ዶ/ር ዮሐንስ ወ/ኪዳን
የህብረተሰብ ጤና ምርምር
ቡድን መሪ

Cc

- ZELEKE ENDALEW
- ETHICAL CLEARANCE COMMITTEE

27 July 2024 12:17 pm

Ref. No. PM23/1024

Date: 4/03/2024

Institutional Review Board (IRB) of St. Paul's Hospital Millennium Medical College (SPHHMC)

Ethical Clearance

Research Titlel: Assessment of determinants and effects of iron-folate supplementation on maternalhematological indices among women attending antenatal care units in public hospitals in Addis Ababa, Ethiopia, 2023.

Principal Investigator Zeleke Endalew

The IRB of SPHMMC has reviewed the above mentioned research proposal and made the following decision:

- Approved:- _____X_____
- Approved with recommendation:- _____
- Approved on condition :-_____
- Disapproved:- _____

The decision is valid for 12 months and the research should be conducted in compliance with the protocol/proposal approved by the IRB of SPHMMC. Any subsequent revision/amendment of the protocol/proposal needs approval before conduct of the research. The researcher should also submit written summaries of the research status to the IRB every 03 months. Upon the conclusion of the study, manuscripts and thesis work to the final/completed research project needs to be submitted to the IRB.

IRB Chair: Gadissa Bedada (PhD)

Research Directorate Director

Signature: _____

Date: March/4, 2024

Cc:

- Vice Provost for Academic and Research
- IRB
- Zeleke Endalew
  SPHMMC

አዲስ አበባ ከተማ አስተዳደር ጤና ቢሮ የሳንባ ምች ልክ ኮምፕረሄንሲቭ ስፔሻላይዝድ ሆስፒታል
ADDIS ABABA CITY ADMINISTRATION HEALTH BUREAUMENELIK COMPREHENSIVE SPECIALIZED HOSPITAL

Date 05/02/16 ዓ.ም

Menelik II Comprehensive Specialized Hospital

MIICSH Training&Research Dep't

To Obstetric & Gynecology Departments of
Menelik II comprehensive specialized hospital

Subject: Requests to conduct ethically approved research

This letter is to support Mr./Ms./Dr./Nr Zeleke Endalew

On the research topic of Assessment of Determinants & Effects of Iron - Folate Supplementation on Maternal Hematological Indices among women attending Antenatal Care units at Menelik II Comprehensive Specialized Hospital Addis Ababa Ethiopia 2023

The study proposal is duly reviewed and approved by Addis Ababa Health bureau IRB and the approval letter is left to our department.

Therefor we request your department and staffs to provide and support for the data collector or principal investigator.

With regards

Research officer Fisseha Tsion Emiru

+251-123 42 72/23 70 15 - 19 ፖ.ሳ.ቁ 7433/4 ኮ ሰ 0111237022 AddiAbaba.Ethiopia

Replying Please quote our Ref.No