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Magnitude and Determinants of Iron Deficiency Anemia among First Antenatal Care Attending Pregnant Women at Selam Health Center, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Kedir Dendir, entitled: **Magnitude and Determinants of iron deficiency Anemia among first antenatal care attending Pregnant Women at Selam Health Center, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviation

AOR - Adjusted odd ratio

ANC - Antenatal care

CBC - Complete blood count

CI - Confidence interval

COR - Crude odd ratio

DDS - Dietary diversity score

HCT - Hematocrit

Hgb - Hemoglobin

ID - Iron deficiency

IDA - Iron deficiency anemia

MCH - Mean corpuscular hemoglobin

MCHC - Mean corpuscular hemoglobin concentration

MCV - Mean cell volume

MUAC - Mid-upper-arm circumference

RBC - Red blood cell

RDW - Red cell distribution width

SF - Serum Ferritin

SST - Serum separator tube

STfR - Soluble transferrin receptor

TBI - Total body iron

Abstract

Background: Iron deficiency anemia during pregnancy is a major global health concern, responsible for approximately 50% of anemia cases worldwide. It is defined as low iron levels in the body. This study points out the need for updated data on iron deficiency anemia and emphasizes understanding factors affecting maternal iron deficiency and its nutritional impact.

Objectives: To investigate the magnitude of iron deficiency anemia and its determinants among first Antenatal Care attending pregnant women at Selam Health Center, Addis Ababa Ethiopia April to May, 2024.

Methods: A prospective, institution-based, cross-sectional study was conducted from April to May 2024 among 170 first antenatal care attendees at Selam Health Centre in Addis Ababa, Ethiopia. Structured questionnaires were used to collect demographic and pregnancy-related data, and blood samples were taken to measure ferritin and hemoglobin levels using specific analyzers, following ethical consent. Data were analyzed using SPSS version 27, with factors associated with iron deficiency anemia assessed through multivariate logistic regression, considering a p-value below 0.05 as statistically significant.

Result: The assessment documented in this research determined that the prevalence of anemia was determined 9.4%(16/170) have a mean Hgb level <11g/dl of , with a specific focus on iron deficiency anemia, which affected 4.1%(7/170) of the participants which have a ferritin level <15µg/l. Iron-deficiency anemia was significantly associated with lower mid-upper arm circumference (AOR = 8.33, 95% CI; 1.23-56.33, P = 0.03), less meal frequency per day (AOR = 5.53, 95% CI; 1.83–36.72, P = 0.046), and dietary diversity score less than five (AOR=7.12, 95% CI; 1-50.71, P = 0.04).

Conclusion: In conclusion, our investigation revealed a low prevalence of iron deficiency anemia. Despite this, factors such as low mid-upper arm circumference, poor dietary diversity, and reduced meal frequency were noted. We recommend educating pregnant mothers on nutrition, and conducting community-based research to further understand these factors.

Key word: Iron deficiency anaemia, pregnant women, Anti natal care, Haemoglobin, and Serum ferritin.

1. Introduction

1.1. Background

Iron serves as a crucial element in hemoglobin molecule (1). It is necessary for the synthesis of hemoglobin, which transports oxygen to the body's tissues (2). The iron cycle in the human body is a complex process that ensures the proper distribution and utilization of iron. The main components of the iron cycle include iron absorption, iron transport, iron utilization, and iron storage. About 1-2 mg of iron is lost every day through skin and enteric desquamation and minor blood losses, which is balanced by intestinal absorption (3). Iron is absorbed in the duodenum and upper jejunum and depends on specific carrier proteins (4). Once absorbed, iron is transported in the bloodstream bound to transferrin, with the majority of iron delivered to the bone marrow for red blood cell production, and lesser amounts going to other tissues for fundamental cellular processes and excess transported to the liver for storage (5).

Iron recycling accounts for most of the iron homeostasis in humans, with most cycling iron coming from the spleen, where red blood cells are destroyed by splenic macrophages, and the iron is recycled to the bone marrow via transferrin (5, 6). Iron (Fe) circulates bound to transferrin, which transferrin receptors then releases into all organs and tissues. The majority of the iron (20–25 mg) that macrophages recycle is given to bone marrow for red blood cells (RBC) synthesis. Duodenal enterocytes absorb 1-2 mg of dietary iron per day; the same amount is lost through blood loss and cell desquamation. The reserve of excess iron is kept in the liver and macrophages(7).

The sequestration of iron in macrophages can also result from iron trapping during infection or inflammation. Hepcidin, a crucial modulator of iron metabolism, has the ability to confine iron within macrophages by inducing the endocytosis and proteolysis of the cellular iron exporter ferroportin. To maintain iron homeostasis and avoid iron overload and deficiency, the body therefore strictly controls the metabolism of iron (8). Beyond this process iron metabolizes abnormally causes iron deficiency and Iron deficiency anemia (IDA). Iron deficiency anemia develops when the body's ability to produce red blood cells is not fully supported by the balance of iron stores, iron intake, and iron loss. Low iron saturation of available transferrin is usually linked to it (9).

During pregnancy, there is a significant increase in iron requirements to support the developing feto-placental unit, enhance the mother's red blood cell mass, and compensate for iron loss during delivery (10). Maternal hepcidin concentrations influence the regulation of iron availability during pregnancy. Hepcidin is an iron-regulatory hormone that produced by the liver. It regulates the distribution of iron in tissues and plasma. The iron balance during pregnancy are fetal iron 270 mg, placental iron 90 mg, baseline maternal body iron loss is 230 mg, maternal RBC mass expansion is 450 mg, total iron requirements during pregnancy are 1040 mg, RBC mass contraction after delivery is 450 mg minus blood lost at delivery (150 − 300) mg, and net pregnancy iron loss to the mother is 740mg (11).

The main cause of IDA is usually due to inadequate intake, poor absorption, or overt or covert blood loss. Iron-deficiency anemia is also largely caused by parasitic infestations in developing nations (12). Starting the right treatment for anemia requires being able to distinguish iron deficiency from other causes of the condition. To help direct the care of these patients, it is also essential to determine the underlying cause of low iron availability (13).

Insufficient iron during pregnancy is primarily attributed to two factors: the woman's iron stores at conception and the amount of iron absorbed throughout gestation. Often, preexisting iron stores are inadequate, and the physiological changes that occur during pregnancy do not sufficiently address the increased iron demands. This is evidenced by the high prevalence of anemia in pregnant women, particularly in developing countries, stemming from these two factors (14). Iron deficiency anemia is present symptoms like Fatigue, weakness, vertigo, irritability, low endurance, hair loss, and dyspnea, which are frequently linked to the physiological changes that occur during pregnancy. Pica (ice craving), restless legs syndrome, poor concentration and work function, increased susceptibility to infection, and cardiovascular stress can cause significant morbidity and reduced quality of life. Because of this, there is an increased risk to the health of mothers, fetuses, and newborns when patients go untreated (15, 16). As a result, this research was initiated to investigate the magnitude and determinants of iron deficiency anaemia among first ANC attending pregnant women in the designated study area.

1.2. Statement of the problem

According to the Global Health Challenges Study 2016, iron deficiency anemia (IDA) is the leading health concern for women and ranks among the top five causes of years lived with disability. While the estimated number of those affected by iron deficiency is at least twice that of IDA, the global prevalence of iron deficiency without anemia remains unclear, making the issue even more critical (17). Over two billion individuals' worldwide, particularly pregnant women, are impacted by nutritional iron deficiency, making it the most prevalent deficiency disease.

The World Health Organization (WHO) 2019 data identifies IDA as a major global health issue in pregnancy, affecting nearly half of pregnant women worldwide. Furthermore, it is estimated that iron deficiency is responsible for approximately 50% of the global burden of anemia. The prevalence of anemia in the global database of the WHO was determined to be 14% based on the regression-based analysis. Recent data indicates that the prevalence of IDA among pregnant women in industrialized countries is 17.4% lower than that in low-income countries, where cases can be up to 56% higher (18).

Prevalence of anemia among pregnant women in Ethiopia has been documented 29%, with IDA representing nearly half of this burden (Central Statistical Agency). According to the Demographic and Health Surveys conducted in East African countries in 2021, a significant 41.82% of pregnant women were reported to have anaemia (19). Despite the fact that the causes are frequently distinct, iron deficiency (ID) and IDA during pregnancy are extremely common throughout the world. Many women do not have enough iron stores at conception to meet the additional needs of pregnancy, which are estimated to be around 1200mg. Pregnant women's iron status can be difficult to assess, but the most accurate diagnostic test currently available is a serum ferritin level of $< 15\mu g/L$ (20).

Investigating iron deficiency anemia in pregnant women is crucial for advancing both maternal and fetal health. Iron deficiency anemia can have profound effects on maternal well-being, leading to increased fatigue, weakened immunity, and complications during delivery. Understanding the causes and effects of IDA is vital for pinpointing at-risk populations and developing preventive strategies. Furthermore, maternal IDA can adversely impact fetal development, leading to low birth weight, premature birth, and cognitive impairments. Researching IDA allows for the early detection of risks that may affect child health, while also informing public health strategies, directing nutritional interventions, and optimizing the use of healthcare resources (21, 22).

Women of reproductive age face a significant risk of anemia and iron deficiency, largely attributable to menstrual blood loss and the heightened iron demands associated with pregnancy (23-25). During pregnancy, inadequate iron levels have been correlated with a greater possibility of detrimental effects on both mothers and their newborns. These outcomes include fatalities among mothers and their infants, preterm delivery, low birth weight, and developmental challenges in children (26-28).

Deepening our understanding of IDA exposure and its implications can result in improved healthcare policies and better health outcomes for future generations. This research highlights significant gaps, particularly the absence of recent data on the prevalence of IDA. There is a need for a deeper understanding of the factors influencing maternal iron deficiency, as well as the associated nutrition, diagnosis, and treatment of IDA. Currently, there is insufficient up-to-date information regarding the prevalence of IDA among pregnant women within the designated research area. This study aims to determine the prevalence of IDA and the factors associated with it to assist ANC services in effectively addressing this issue through appropriate care and treatment. The results will provide essential data to support the interventions carried out within the healthcare facility (21, 22).

1.3. Significance of the study

The purpose of this study was to determine the prevalence of Iron deficiency anemia and its determinants among first antenatal care visit pregnant women in Selam Health Center. This was enable for early interventions and follow up of pregnant women and helps health care providers, policy makers and other stake holders to ascertain the depth of the situation and design intervention roadmap.

2. Literature review

In the year 2012, a study conducted in Yugoslavia revealed that 31.7% (149 out of 470) of the subjects had reduced iron levels, indicated by ferritin levels below 20 μg/l. Additionally, 18.5% (87 out of 470) were diagnosed with anaemia, characterized by hemoglobin levels falling below 110 g/l. The prevalence of iron deficiency anemia is significantly higher among females from developing countries and the former Yugoslavia, with p-values of 0.012 and 0.004, respectively. Additionally, a substantial decrease in iron levels was observed in patients over the age of 30, with a p-value of 0.018 (29).

According to a cross-sectional investigation performed in Singapore, 2019, moderate and severe iron depletion were present in 660 (67.0%) and 67 (6.8%) of the women, respectively. Women with severe iron deficiency had higher plasma sTfR (soluble transferrin receptor) than those with sufficient iron levels (median 17.6 versus 15.5nmol/L; $p < 0.001$). The following factors were linked to higher odds of mild and severe iron depletion: age < 25 years (odds ratio 2.36; 95% confidence interval 1.15–4.84), university qualification (1.64; 1.13–2.38), multiparty (1.73; 1.23–2.44), and absence of iron-containing supplementation (3.37; 1.25–8.53) (30).

A study conducted in Thailand in 2006 revealed that iron deficiency (ID), iron deficiency anemia (IDA), and other forms of anaemia were observed in 34.4%, 37.8%, and 7.8% of the 180 pregnant women enrolled in the study, respectively. Stool sample analysis revealing prevalence rates of Ascaris, Trichuris, and hookworm at 47%, 48%, and 25%, respectively (31).

According to a systemic review, the prevalence of iron deficiency was 31.6 to 34.6% in Malaysia in 2022, while the overall prevalence of anemia in pregnancy ranged from 19.3–57.4%. Extremes of reproductive age, late prenatal booking, non-adherence to hematinic, Indian ethnicity, low maternal educational attainment, low family income, and unemployment were all significantly linked to anemia during pregnancy (32).

A study carried out in Finland in 2003 revealed that anaemia was observed in 2.6% of the cases, with 0.3% of these instances occurring during the first trimester. When confounding factors were taken into account, anemia found in the initial trimester was linked to below-average birth weight babies (OR = 3.14, 95% CI; 1.35 – 7.28), but there were no appreciable differences in the

outcomes between the anemia groups in the mid- and third trimesters and the non-anemic women. First trimester anemia did not significantly correlate with either an early delivery of less than 37 weeks (AOR=1.80, 95% CI: 0.72–4.49) or a small birth weight for gestational age (AOR=0.98, 95% CI: 0.41–2.17) (33).

In UK, 2016 based on ferritin, sTfR, and TBI (Total Body Iron), incidence of ID among the 4420 females was 19.6, 15.3, and 15.7%, respectively. Depending on which iron parameter was used, risk factors for ID included high maternal body weight, multi-parity, socioeconomic disadvantage, and inflammation in addition to maternal age under 25. Reduced risk of gestational diabetes mellitus was linked to ID, which was defined by serum ferritin and TBI but not sTfR. There was an increased risk of large-for-gestation-age infants when ID was defined using TBI alone (34).

Linking Hemoglobin and plasma ferritin measurements during the initial trimester, alongside obstetric and perinatal data sourced from a hospital database, a prospective cohort study was carried out in Denmark, involving 5763 women who are pregnant with a single fetus of which 1.2% had IDA, and 14.2 % had non-anemic low iron levels. IDA women were potential to development of diabetes linked to pregnancy (AOR, 3.8, 95% CI, 1.4–9.0). The likelihood of stillbirth was significantly increased (AOR 4.0 95%CI 1.0 – 14.3) relative to those who were iron-replete and non-anaemic women. After intensified iron supplementation, 81.5% and 67.7% of group 1 and 2 remained iron-deficient (35).

An investigation conducted in Belgium in 2013 approximately 40% of women in their third trimester and 6% of women in their first had Serum ferritin (SF) levels below 15 μg/L. A little over 21% of women in their third trimester and 4% of those in their first had anemia (Hb <110 g/L). Of the women in their third trimester, 16% had iron-deficiency anemia (SF <15 μg/L & Hb <11.0 g/L), 23% were non-anemic status accompanied by iron deficiency, and roughly 7% had tissue iron deficiency (sTfr >8.5 mg/L) (36).

A Portuguese study conducted in 2016 there were 201 women in total, five of whom (2.49%) had anemia. Furthermore, 22 (10.9%) had severe iron depletion, and 77 (38.3%) had iron deficiency. The only risk factor found was the age of the mother. For women under the age of twenty (AOR 12.99, 95% CI 2.41 - 70.0) and for women over thirty (AOR 2.09, 95% CI 1.05 - 4.14) (37).

In South India in 2001, the percentage of women in their first, second, and third trimesters who had anemia (Hb <11 g/dl) was 56.6%, 70.2%, and 69.5%, respectively. The third trimester women had significantly ($P < 0.05$) higher rates of iron deficiency (SF <12 µg/L) than the first trimester women. Given the high prevalence of anemia in every trimester of pregnancy, iron supplementation should begin as early as the fourth month of pregnancy (38).

A cross-sectional research undertaken in Turkey, 2021 involved 165 pregnant women. The findings revealed that 65.5% of the participants exhibited iron deficiency, while 15.2% were diagnosed with anaemia. Key factors associated with anaemia included age, the number of previous births, the consumption of folic acid supplements, dietary folate equivalents, and overall iron intake. Additionally, total intake levels of iron, phosphorus, vitamin B1, and vitamin B2 were identified as significant contributors to iron deficiency (39).

In 2016, a cross-sectional investigation undertaken at Liaquat University in Jamshoro, Pakistan, focusing on 305 pregnant women diagnosed with iron deficiency anaemia. The findings revealed that a significant proportion of these women were young, with 170 (55.73%) aged between 20 and 30 years. Most participants belonged to a low socioeconomic status, with 254 (83.27%) being multiparous and 104(34.09%) of the women presented with critically low hemoglobin level. The study highlighted a considerable risk of complications among these women, including antepartum hemorrhage (16.06%), renal failure (15.73%), disseminated intravascular coagulation (17.70%), and a total of 16 fatalities (5.24%) (40).

In the year 2015, a study of a cross-sectional design was performed in Iran, encompassing 418 pregnant women. The findings indicated that the prevalence of IDA was 22.5% during the first trimester (confidence interval: 18.4%–26.5%) and 45.9% in the third trimester (confidence interval: 41.1%–50.6%). A significant correlation was observed between pregnancy interval and IDA in both the first and third trimesters ($P < 0.009$ and $P < 0.001$, respectively) (41).

A study of a cross-sectional design was executed in 2022; encompassing 389 expectant receiving ante-natal cares at public hospitals in Côte d'Ivoire revealed that 25.8% of the participants exhibited iron deficiency, while 30.4% were diagnosed with iron deficiency anaemia. The adjusted odds ratios (AORs) identified several independent and significant predictors of iron deficiency anaemia, including low dietary diversity (AOR 8.35), multiparty (AOR 13.18), meal skipping (AOR 3.05), inadequate intake of energy (AOR 5.369), protein (AOR 2.74), and vitamin C (AOR 2.43). Additionally, the study found that being in the 2nd and 3rd stages of pregnancy significantly increased the harm (AOR 6.04 and 4.18, respectively) (42).

Systemic review conducted in Nigeria in 2020 reveals, It has been reported that a high percentage of pregnant women suffer from iron deficiency anemia. It is important to promote family planning, educate people about its benefits, and ensure that antenatal care services are used appropriately. Women should be given more economic power, and health facilities should be built in places where they are not present to promote early booking and use of antenatal care services (43).

According to a cross-sectional study, 12.3% of Nigerians had IDA in 2020. A minimum 2-year inter-pregnancy interval (AOR; 0.20 95%CI 0.05 – 0.97 P = 0.021) and routine prenatal iron supplementation (AOR; 0.18 95%CI 0.07 – 0.46 P = 0.001) have been found to be significantly associated with IDA. Iron deficiency anaemia (IDA) remains a prevalent concern among pregnant women in Lagos, primarily associated with maternal perinatal health challenges (44).

The meta-analysis included 10, 281 pregnant women from 20 studies in Ethiopia, the combined incidence of anaemia in expectants in Ethiopia was 31.66% (95% CI (26.20 - 37.11)). The Amhara region had the minimal occurrence of anaemia in expectants (15.89% (95% CI 8.82, 22.96)), while the Somali region had the highest prevalence (56.80% (95%CI 52.76 - 60.84)). The results of the aggregated extent observed in the subgroup evaluation revealed that anemia was less common in Primigravida (AOR: 0.61, 95% CI 0.53 - 0.88) and urban (AOR: 0.73, 95% CI 0.60 - 0.88) women. On the flip side, mothers who had malaria infection during pregnancy (AOR; 1.94, 95% CI 1.33 - 2.82), and, compact duration between pregnancies (AOR; 2.14, 95% CI 1.67 - 2.74) were more likely to suffer from anaemia (45).

The investigation comprised a cross-sectional study executed in Woldiya, Ethiopia, 2016, it was found that 95 out of 243 women (39.1%) enrolled in the research were anaemic. The prevalence of anaemia was particularly pronounced in the first (52.2%) and second (52.6%) trimesters. Among the participants, 86 (90.5%) were identified as having mild anaemia, while nine (9.5%) were categorized as having severe anaemia. The findings also indicated that pregnant women with a history of antenatal care follow-up had a lower prevalence of anaemia (48.2%), compared to a much higher prevalence (94.4%) among those without such follow-up (46).

Cross-sectional research design was implemented in Gondar Ethiopia, 2019 revealed that within 217 enrolled women, 28 (12.9%) were diagnosed with anaemia. The distribution of anaemia types among these women indicated that 75% experienced mild anaemia, 21.4% had moderate anaemia, and 3.6% suffered from severe anaemia. Notably, the incidence of anemia was higher at initial trimester (21%) and the third trimester (17.9%). Furthermore, 25% of the anaemic pregnant mothers were found to have IDA, contributing to an overall extent of IDA 3.2% (7 out of 217 participants) (47).

In the Wolayita zone of Ethiopia, a cross-sectional study was performed in 2023, selecting 270 participants through a multistage sampling process. The study gathered data using hemoglobin, serum ferritin, and C-reactive protein as measurement tools. Iron-deficiency anaemia was present in 11.3% of cases (95% CI: 8.9–15.7).

Several determinants were discovered to be connected with IDA. Pregnant mother who didn't have ANC follow-up showed a threefold increase in likelihood to experience IDA in contrast those who did receive such follow-up during their recent pregnancy (AOR = 3, 95% CI: 1–8.9). Moreover, both nutritional status and mid-upper arm circumference (MUAC) exhibited a strong correlation with the occurrence of IDA among expectant mothers (AOR = 4, 95% CI: 1.5–10.8). Additionally, having less than two years between successive pregnancies was tied to fourfold rise in likelihood to develop IDA (AOR: = 4, 95% CI: 1.2–12.4) (48).

A cross-sectional analysis conducted at Saint Paul Hospital in Ethiopia in 2018 revealed that the prevalence of anemia among the surveyed population was 11.6% (95% CI: 7.8%-14.8%). The findings indicated that pregnant women in their second and third trimesters had a significantly higher risk of anemia compared to those in their first trimester (AOR: 6.72, 95% CI: 1.17-38.45, p-value = 0.03). Additionally, the results showed that pregnant women who did not receive iron or folic acid supplementation had an increased likelihood of developing anemia (AOR: 4.03, 95% CI: 1.49 - 10.92, p-value = 0.01) compared to those who did (49).

A 2015 study at St. Paul Hospital in Ethiopia found that maternal IDA impacts newborn iron stores. Mothers with IDA had median hemoglobin and ferritin levels of 12.2g/dL and 47.0ng/mL, respectively, while newborns had levels of 16.2 g/dL and 187.6ng/mL of the 89 mothers, 21 (23.6%) were classified as IDA, and the remaining 68 (76.4%) as non-anemic (NA). Newborns of IDA mothers had significantly lower serum ferritin (P = 0.017) and hemoglobin levels (P = 0.024). Significant correlations were also observed between newborn and maternal hemoglobin and ferritin levels (P values < 0.05) (50).

3. Objectives

3.1. General objective

- To assess magnitude of iron deficiency anemia with its determinants among first ANC attending women at Selam Health Centre, Addis Ababa, Ethiopia, April to May, 2024.

3.2. Specific objectives

- To assess magnitude of iron deficiency anemia among first ANC attending women at Selam Health Center, Addis Ababa, Ethiopia April to May, 2024.

- To identify factors associated with iron deficiency anemia among first ANC attending women at Selam Health Center, Addis Ababa, Ethiopia April to May, 2024.

4. Method and Materials

4.1. Study area

The study was undertaken in Selam Health Center situated in Addis Ababa, Ethiopia. Addis Ababa was found at the center of the nation characterized by its latitude of 9o2'N and longitude of 38o45'E with an elevation of 2446m. The total land area covered was 540 square kilometers (51) with population size of above 5.7 million of which 2.8 million are males and the remaining are females(52).

The health center was established in 1946 to provide health services to communities that are frequently underserved. It handles an average of 200 emergency and outpatient clients each day. The ANC department offers a range of services, including prenatal check-ups, screening tests, health education, and counseling to assist pregnant women. The department is staffed by a multidisciplinary team that includes physicians, midwives, and nurses. Patient care emphasizes monitoring the progress of pregnancies, managing risks, and providing support. The facilities are designed to create a comfortable environment for pregnant mothers, featuring consultation rooms, examination areas, ultrasound facilities, and spaces for health education. (53).

4.2. Study design and period

A cross-sectional institutional study was conducted from April to May, 2024.

4.3. Population

4.3.1. Source population

The source populations of the study were all pregnant mothers who were attending at Selam Health Center within the timeframe of the research.

4.3.2. Study population

The study units were all first ANC contact attending pregnant women, who were attending at Selam Health Center Between April, 2024 to May, 2024.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

- Pregnant mothers who attended their first ante-natal care follow-up and provided informed consent were participated in the research.

4.4.2. Exclusion criteria

- Pregnant women on a course of iron therapy.
- Pregnant women with chronic infections or inflammation (C-reactive protein positive) were excluded.

4.5. Sample size calculation and sampling technique

4.5.1. Sample size calculation

The number of individuals included in the study was determined to be considering a 11.3% prevalence of IDA in a recent similar study done in Ethiopia using the standard statistical calculation with the 95% confidence level (48).

Then following formula is used to calculate the sample size.

$$N = \frac{(Z\alpha/2)\,2\,(pq)}{d^2}$$

$$N = \frac{(1.96)\,2\,(0.113 \times (1-0.113)}{0.05^2}$$ **= 154,** Adding Non response rate =10% ≈ **170**

Where, N = the desired sample size

P= Prevalence

Z = standard normal value at 95% CI=1.96

d = 5%=0.05 - the absolute precision

4.5.2. Sampling technique

Non probability convenient sampling techniques were applied to select the first ANC pregnant mothers.

4.6. Study variables

4.6.1. Dependent variable

Magnitude of Iron deficiency Anemia

4.6.2. Independent variable

- **Socioeconomic and Demographic factors:** Age, Occupation, education, Residence and Family size.
- **Clinically related factors:** Intestinal parasitic infection, Morning sickness, chronic infection and dental problem.
- **Obstetrics factors and gynecological factors:** history of abortion, gravidity and trimester.
- **Nutritional factors:** Number of meal consume per day, MUAC, and Dietary diversity.

4.7. Operational definitions

IDA: Those pregnant women having serum ferritin level < 15 µg/l and haemoglobin level <11g/dl (54).

MUAC: Undernourished (MUAC of < 23 cm) and normal (MUAC ≥23 cm) (55).

Inadequate dietary diversity score (DDS): A pregnant woman consumed fewer than five of the ten food groups in the 24 hours leading up to the survey (56).

Adequate dietary diversity score (DDS): A pregnant individual had five or more varied food groups from a set of ten in the 24 hours prior to the survey (56).

4.8. Measurements and data collection

Before data collection, all necessary structured questionnaires in both open and closed-ended formats were prepared. The subjects were informed about the goals of the research, and interviewers translated the materials into local languages to enhance understanding and minimize bias during data collection. Study information was gathered through personal interviews, and some clinical data were reviewed from the patients' medical cards.

The Mid-Upper Arm Circumference (MUAC) is a quick and straightforward tool used to assess the nutritional well-being of expectant mothers. The cut-off values for MUAC can differ widely across regions and depend on factors such as the population, age of pregnant women, and gestational week. For women in early pregnancy, the cut-off points for detecting underweight and obesity are set at 24.0 cm and 29.0 cm, respectively, both showing good predictive values. In late pregnancy, the corresponding cut-off points are 23.0 cm for underweight and 28.0 cm for obesity. The measurement is taken at the midpoint between the acromion and olecranon processes, located on the shoulder blade and ulna of the arm, respectively (57, 58).

The assessment of dietary diversity (DD) utilized a standardized tool provided by the Food and Agriculture Organization of the United Nations (FAO) as outlined in its 2019 guidelines for pregnant women. The evaluation was conducted using the 24-hour recall method, where participants were asked about their consumption of food items from a predetermined set of 10 Minimum Dietary Diversity for Women (MDD-W) food groups. Subsequently, the dietary diversity score (DDS) was calculated out of a possible 10, in accordance with FAO guidelines (48).

4.8.1. Specimen collection, processing and analysis

The specimens were collected from the gynecology ANC department at Selam Health Centre. Specifically, 4 ml of venous whole blood was drawn in the morning, with 2 ml placed in an EDTA tube and 2 ml in an SST tube, along with a stool sample. The analysis of these specimens included measuring ferritin levels using the Cobas-2600 hormone analyzer, a device produced by Roche Diagnostics in Switzerland. Hemoglobin levels were assessed using the Mindray CBC analyzer, manufactured by Mindray Biomedical Electronics Ltd in China. Additionally, the stool sample was subjected to microscopic examination for intestinal parasites, and C-reactive protein levels were assessed for screening purposes by trained laboratory professionals at the Selam Health Center.

4.8.2. Laboratory Analysis

4.8.2.1. Hemoglobin test principle

Hemoglobin levels were measured with the Mindray BC 3000 Plus hematology analyzer, a device manufactured by Mindray Bio-Medical Electronics Co., Ltd. in China. Trained laboratory professionals performed the analysis at the Selam Health Center laboratory. The determination of hemoglobin is carried out using the colorimetric method. In this process, the WBC/HGB dilution is introduced into the WBC bath, where it is mixed with a specific amount of lyse.

This converts hemoglobin into a measurable hemoglobin complex at a wavelength of 525 nm. A LED, positioned on one side of the bath, emits a beam of light that passes through the sample and through a 525 nm filter before being detected by a photo-sensor on the opposite side. The resulting signal is then amplified, and the voltage is measured and compared to a blank reference reading (obtained when only the diluent is present in the bath). Hemoglobin (HGB) levels are calculated, and the final results are reported in g/L. (Mindray BC 3000 Plus Standard Operating Procedure).

4.8.2.2. Principle of Cobas-e 411 to measure serum ferritin

Serum ferritin levels were measured using the Cobas e 411 hormone analyzer, a device produced by Roche Diagnostics, based in Switzerland. The analysis was conducted by trained laboratory professionals at the accredited Ethiopian Public Health Institute laboratory.

During the first incubation, which lasted for nine minutes, a sandwich complex was created by combining 6µL of a sample with a monoclonal antibody that was specific to ferritin and marked with ruthenium and biotin. The complex was coupled to a solid phase by the interaction of biotin and streptavidin following the addition of streptavidin-covered micro-particles. A measurement cell was filled with the reaction mixture, and the electrode surface magnetically captured the micro-particles. In order to measure the chemiluminescence emission brought on by applying voltage to electrodes, a photomultiplier was used (59).

4.8.2.3. CRP test principle

C-reactive protein (CRP) is measured using latex-enhanced agglutination method. The basic principle of particle-enhanced assays is the interaction of a soluble analyte with the matching antigen or antibody attached to polystyrene particles. Particles with a polystyrene core and a hydrophilic shell are used to covalently link anti-CRP antibodies in order to quantify CRP. Latex particles coated with mouse monoclonal anti-CRP antibodies are combined with a diluted solution of the test sample. The test sample's CRP will combine with the latex particles to form an antigen-antibody complex (60).

4.8.2.4. Stool examination test principle

The stool sample is macroscopically analyzed for color, consistency, quantity, form, odor, and the presence of mucus. For the saline wet mount examination, the stool is emulsified in normal saline, and a large drop is placed on a glass slide, which is then covered with a cover slip. The sample is subsequently examined under a light microscope to detect any intestinal parasites (61).

4.9. Data quality assurance

Throughout the approach utilized for the collection of data, questionnaires were developed with a unique ID number that aligned with the specimen ID number. After the interview had been completed, thorough cross-checking was conducted every day, steps are undertaken to assure the entirety of collected information.

In order to guarantee the quality of laboratory findings, all protocols were strictly adhered to, following Standard Operating Procedures (SOP) and Internal Quality Control (IQC) guidelines. Test accuracy was ensured by adhering to safety measures across pre-analytical, analytical, and post-analytical phases.

- **Pre-analytical phase:** Proper sample collection, transportation, and processing procedures were applied based on standard operating procedures.
- **Analytical phase:** Quality control materials were tested prior to analyzing the samples, and analysis proceeded only if the control test results met the required standards. All control materials were updated and provided by the manufacturer, and their handling was

conducted by trained laboratory professionals in each laboratory. For the Mindray BC 3000 Plus, hematological controls were utilized to verify the reliability of the hemoglobin results, and similar way were employed for the ferritin and C-reactive protein tests.

- **Post-analytical:** The findings were documented clearly in legible handwriting on the data collection sheet. The results were interpreted according to the reference range.

4.10. Data interpretation and Analysis

The collected data was entered into SPSS software version 27 for analysis. The analyzed factors associated with IDA were conducted using both bi-variable and multivariable logistic regression techniques. Variables that exhibited p-value < 0.25 in bi-variate tests were deemed suitable nominees for inclusion in the multi-variable analysis to account for potential confounding effects. Once the relevant variables were identified, the multivariable analysis was conducted, incorporating all selected variables. The goodness-of-fit evaluation based on the Hosmer and Lemeshow approach was undertaken, whether the assumptions necessary for multiple logistic regressions were satisfied.

The adjusted odds ratios (AOR) and their respective 95% CI were calculated to assess the strength of the associations. All statistical tests were two-tailed, with a significance level set at p value < 0.05. The findings are subsequently presented through narrative descriptions and descriptive statistics, including tables.

4.11. Ethical Consideration

The Department Research and Ethical Review Committee (DRERC) of the Medical Laboratory Sciences Department, College of Health Sciences, Addis Ababa University, granted ethical approval for this research under protocol number DRERC/757/24/MLS/.

Following the endorsement by the research review committee, the objective of the study was conveyed to the health center through a letter of support from the College of Health Science department of medical laboratory sciences. Afterward, written authorization was received from the Health center. Prior to participation, each participant granted their informed consent involved in the research. To uphold confidentiality, the data collection form did not contain any personal

identifiers. The information collected was only accessible to the principal investigator, who ensured its confidentiality and stored it in a secure environment.

4.12. Dissemination of result

The findings will be presented to the Department of Medical Laboratory Sciences at the College of Health Sciences, Addis Ababa University. Additionally, the results will be shared with the governing body of Selam Health Center, the ANC department, and other relevant stakeholders through various channels, including reports, direct mail, conference presentations, and seminars, to facilitate the implementation of the research recommendations. Furthermore, the research will be submitted for publication in reputable journals. The outcomes will also be showcased at both national and international conferences.

5. Result

5.1. Socio-demographic characteristics

Out of the 170 participants in the research, the majority, 111 individuals (65.3%), were aged between 15 and 24 years. In terms of education, 149 participants (87.6%) were having a formal education. The data indicated that most participants, 153 individuals (90%), lived in urban environments (Addis Ababa), while the rest were situated in rural area (sululta, and sansusi oromia regions). Furthermore, 125 participants, or 73.5%, belonged to families with more than five members (Table; 1).

Table 1: Socio-demographic characteristics of study participants in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	**Categories**	**Frequency**	**Percentage (%)**
Age (yrs.)	15– 24	39	22.9
	25 – 34	111	65.3
	≥ 35	20	11.8
Occupation	Government employee	15	8.8
	Private employee	45	26.5
	Merchant	5	2.9
	Housewife	89	52.4
	Daily laborer	6	3.5
	Student	10	5.9
Education	Informal	21	12.4
	Formal	149	87.6
Residence	Rural	17	10
	Urban	153	90
Family size	< 5	125	73.5
	≥ 5	45	26.5

5.2. Obstetrics factors and gynecological characteristics

In our study, it was found that 128 participants, representing 75.3%, possessed information regarding anemia. Additionally, 123 participants, or 72.4%, were classified as multigravida. Among the participants, 39 individuals, which is 22.9%, reported a history of abortion within the last year, and 121 participants, equating to 71.2%, were in their first trimester of pregnancy (Table 2).

Table 2: Obstetrics and gynecological factors among pregnant women in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	Categories	Frequency	Percentage
Previous history of abortion (in the last year)	Yes	39	22.9
	No	131	77.1
Information about anemia	Yes	128	75.3
	No	42	24.7
Have child before this pregnancy	Yes	111	65.3
	No	59	34.7
Number of children	≤2	144	84.7
	> 2	26	15.3
Morning sickness	Never	50	29.4
	Rarely	44	25.9
	Sometimes	38	22.4
	always	38	22.4
Gravidity	Primigravida	47	27.6
	multigravida	123	72.4
Trimester	First	121	71.2
	Second	20	11.8
	Third	29	17.1

5.3. Nutrition-related characteristics and feeding habit

In our study, the majority of the participants, totalling 126 (74.1%), had access to dietary information. Out of the 170 pregnant women included in the study, 145 (85.3%) reported eating meals three or more times a day, in contrast to 25 (14.7%) who consumed fewer than three meals daily.

Among the participants, 45 (26.5%) were identified as having food allergies. Additionally, only 11 participants (6.5%) had medical conditions that affect their eating habits. The analysis also revealed that 36 participants (21.2%) consumed a limited variety of foods, defined as fewer than five food groups, whereas 134 participants (78.8%) exhibited a sufficient diversity in their dietary intake (Table 3).

Table 3: Nutrition-related characteristics and feeding habit among pregnant women in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	**Categories**	**Frequency**	**Percentage (%)**
Having dietary information	Yes	126	74.1
	No	44	25.9
Food allergy	Yes	45	26.5
	No	125	73.5
Medical condition that affects eating habit	Yes	11	6.5
	No	159	93.5
Dental problem that affects eating habit	Yes	14	8.2
	No	156	91.8
Meals consumed per day	Less than three	25	14.7
	Three and more	145	85.3
dietary diversity score (DDS)	Adequate	134	78.8
	Inadequate	36	21.2

5.4. Hematological profile among first ANC pregnant mothers

In our study, pregnant mothers diagnosed with IDA exhibited significantly lower mean values for RBC, HCT, Hgb, and MCH compared to pregnant mothers without IDA. Specifically, the mean RBC value was 3.12 ± 0.36 versus 4.13 ± 0.43 ($p < 0.001$), the mean HCT value was 27.60 ± 3.67 versus 37.97 ± 3.58 ($p < 0.001$), the mean Hemoglobin value was 9.40 ± 1.11 versus 12.35 ± 1.12 ($p < 0.001$), and the mean MCH value was 23.50 ± 2.69 versus 30.29 ± 2.16 ($p < 0.001$). On the contrary, pregnant mothers with IDA had a higher mean RDW value (16.11 ± 0.91) compared to those without IDA (14.35 ± 0.69, $p < 0.001$) (Table 4).

Table 4: Hematological Profile of First ANC Pregnant Women at Selam Health Center, Addis Ababa, Ethiopia, 2024 (n = 170).

Variables	IDA	Not IDA	P-value
	Mean ±SD	Mean ±SD	
Red blood cell (cells/µL)	3.12 ± 0.36	4.13 ± 0.43	**< 0.001**
Hemoglobin(g/dL)	9.40 ± 1.11	12.35 ± 1.12	**< 0.001**
Hematocrit (%)	27.60 ± 3.67	37.97 ± 3.58	**< 0.001**
Mean Cell Volume (fL)	88.24 ± 1.63	92.11 ± 5.69	0.075
Mean Corpuscular Hemoglobin (pg)	23.50 ± 2.69	30.29 ± 2.16	**< 0.001**
Mean Corpuscular Hemoglobin Concentration (g/dL)	33.07 ± 0.93	32.28 ± 1.22	0.093
Red cell Distribution Width (%)	16.11 ± 0.91	14.35 ± 0.69	**< 0.001**

5.5. Magnitude of iron deficiency anaemia

The analysis showed that anemia prevalence was 9.4% (16 out of 170 participants), with a particular emphasis on iron deficiency anemia, which affected 4.1% (7 out of 170 participants). Furthermore, 34 participants, representing 20%, were classified as undernourished, as indicated by a Mid-Upper Arm Circumference (MUAC) measurement of less than 23 cm. additionally, 16 women (9.4%) exhibited low hemoglobin levels, qualifying them as anemic, while 7 participants (4.1%) displayed low ferritin levels indicative of iron deficiency anemia. The stool examinations revealed that 9 participants (5.35%) were infected with intestinal parasites, including 11.1% with Hymenolepis *nana*, 33.3% with Giardia *lamblia*, and 55.6% with Entamoeba *histolytica* (Table 5).

Table 5: Clinical-related and other factors among pregnant women in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	Categories	Frequency	Percentage
MUAC (cm)	23 cm and above	136	80
	Less than 23 cm	34	20
Hemoglobin (Hg) level (g/dl)	Low	16	9.4
	Normal	154	90.6
Ferritin level (µg/L)	Low	7	4.1
	Normal	163	95.9
Intestinal parasite infection	Negative	161	94.7
	Positive	9	5.3
Iron deficient anaemia/IDA	Yes	7	4.1
	No	163	95.9

Footnote: MUAC (Mid-Upper Arm Circumference) measurements were utilized to assess the nutritional status of pregnant mothers. Participants were classified as undernourished if their MUAC was less than 23 cm, while values between 23 cm and 28 cm were considered normal, and values greater than 28 cm indicated obesity. Hemoglobin levels were categorized as anemic when below 11 g/dL, with values above this threshold considered normal. Ferritin levels were regarded as low and indicative of iron deficiency if they were below 15µg/L, while values above this level were deemed normal.

Table 6 illustrates that bivariate logistic regression analyses were performed to identify the independent factors of IDA among expectants. The factors like low MUAC, less frequency of meals per day, previous history of abortion, and low dietary diversity score were found to be candidates for multivariate logistic regression (Table 6).

Table 6. Bivariate binary logistic regression analysis on factors associated with iron deficiency anemia (IDA) among pregnant women in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	Categories	IDA		COR (95%CI)	P value
		Yes (n=7)	No (n=163)		
Information about anemia	Yes	4	124	1	0.271
	No	3	39	2.39 (0.51-11.12)	
Child before	Yes	4	107	0.698 (0.15-3.23)	0.645
	No	3	56	1	
Number of children	≤ 2	5	139	1	
	> 2	2	24	2.32 (0.43-12.63)	0.332
Family size	< 5	4	121	1	0.326
	≥ 5	3	42	2.16 (0.46-10.05)	
Gravidity	Primigravida	2	45	1	
	multigravida	5	118	0.95 (0.18-5.09)	0.955
Trimester	First	2	119	1	
	Second	2	18	6.61 (0.88-49.92)	0.670
	Third	3	26	6.87 (1.09-43.18)	0.40
Having dietary information	Yes	4	122	1	0.306
	No	3	41	2.23 (0.48-10.39)	
Food allergy	Yes	3	42	2.16 (0.47-10.05)	0.326
	No	4	121	1	
Chronic infection or continues medication that affects eating habit	Yes	1	10	2.55 (0.28-23.28)	0.407
	No	6	153	1	

Dental infection that affects eating habit	Yes	1	13	1.92 (0.22-17.21)	0.559
	No	6	150	1	
MUAC (cm)	23 and above	2	134	1	
	< 23	5	29	11.6 (2.14 – 62.5)	0.005 [a]
Frequency of meal per day	< 3 times	3	22	4.8 (1-22.94)	
	≥3 times	4	141	1	0.049 [a]
Previous history of abortion	Yes	4	35	4.9 (1.04 -22.8)	
	No	3	128	1	0.044 [a]
Dietary diversity score (DDS)	Adequate	2	132	1	0.006 [a]
	Inadequate	5	31	10.65 (1.97 -57.45)	

[a] =variables exported to multivariate logistic regression; 1 = reference groups.

In the multi-variable logistic regression assessment, factors such as MUAC, frequency of meals consumed per day, and DDS were found to be statistically significant in relation to IDA, indicated by a p - value below 0.05, and a 95% CI (Table 7).

A pregnant woman with a MUAC under 23 cm were 9.84 folds considerably more susceptible to the development of IDA relative to MUAC measurements of 23 cm or above (AOR = 9.84; CI: 1.60–60.46). This investigation revealed that pregnant women who ate fewer than three meals per day and had a low dietary diversity score had 4.98 and 9.13 increased likelihood of developing IDA in comparison to those who consumed three or more meals per day and had an adequate DDS (AOR = 4.98; CI: 1.79–31.33; AOR = 9.13; CI: 1.36–61.48), respectively (Table 7).

Table 7: Multivariate analysis of variables associated with iron deficiency anemia (IDA) among pregnant women in Selam Health Center, Addis Ababa, Ethiopia, 2024 (n =170).

Variables	Categories	Iron Deficiency Anemia (IDA)		COR (95%CI)	AOR (95%CI)	P -value
		Yes (n=7)	No (n=163)			
MUAC (cm)	23 and above	2	134	1	1	
	< 23	5	29	11.6 (2.14 – 62.5)	9.84 (1.60 – 60.46) *	**0.014**
Frequency of meal per day	< 3 times	3	22	4.8 (1-22.94)	4.98 (1.79 – 31.33) *	
	≥3 times	4	141	1	1	**0.047**
Previous history of abortion	Yes	4	35	4.9 (1.04 -22.8)	1.87 (0.32 -11.09)	
	No	3	128	1	1	0.491
Dietary diversity score (DDS)	Adequate	2	132	1	1	**0.023**
	Inadequate	5	31	10.65 (1.97 -57.45)	9.13 (1.36-61.48) *	

Abbreviations: MUAC - Middle upper arm circumstance, *P < 0.05 and 95% CI = confidence interval.
1 = reference groups.

6. Discussion

6.1. Prevalence of iron deficiency anaemia

Among micronutrient deficiencies, iron deficiency stands out as the most frequently occurring globally (62-64) and is acknowledged as the leading cause of anaemia, accounting for roughly 25-50% of all anaemia cases. According to a report by the WHO, nearly half of all anaemic pregnant women suffer from IDA, a finding that aligns with the results of this study (65, 66).

Our study reveals that the overall prevalence of anemia is 9.4%, with iron deficiency anemia accounting for 4.1% of this figure. In contrast, a cross-sectional study conducted by Gebreweld A *et al*. in 2018 and Terefe B *et al*. in 2015 at St. Paul Hospital reported anemia and iron deficiency anemia prevalences of 11.9% and 23.6%, respectively. Their analysis showed that pregnant women in the second and third trimesters had a significantly higher likelihood of experiencing anemia compared to those in their first trimester (49). This discrepancy may explain why our findings are lower, as the majority of participants in our study were in their first trimester.

Our study finding was comparable to those of Enawgaw B *et al* in 2019, who reported an overall prevalence of iron deficiency anaemia at 3.2% (7 out of 217 subjects) (47). Additionally, our research aligns with the findings done in 2021 by Pobee RA *et al*, in Ghana which reported a 6% incidence of IDA. Their work indicated that the extent of IDA was more pronounced during the second and third trimesters in comparison to the first trimester (67). These factors may be relevant to our investigation, where the lower prevalence observed can be attributed in light of the truth that most of our study units were in their first trimester.

On the other hand, the prevalence of iron deficiency anaemia observed in our investigation is somewhat lower from the findings noted in 2014 by Gebremedhin S et al. in rural Sidama, Southern Ethiopia, as well as those reported by Laelago et al. in Wolayita Sodo, Ethiopia, where the rates were found to be 8.7% (68) and 11% (48), respectively.

The differences noted can be ascribed to various socio-demographic factors, nutritional habits, and variations in healthcare access, sample size differences, and the occurrence of other diseases that may lead to IDA. The lower incidence of IDA observed may be due to most participants

living in urban settings with better nutritional access. These urban settings may foster a more supportive environment for the health and nutritional well-being of the participants. Improved dietary conditions can enhance iron bioavailability, which may influence the prevalence of IDA during pregnancy (69).

6.2. Associated risk factor with iron deficiency anaemia

The nutritional status of women demonstrated statistically important connection with the initiation of IDA. Women presenting with MUAC of less than 23 cm, indicative of inadequate nutritional status, were found to be 9.84 folds more probable to develop IDA in contrast to their counterparts. This situation may arise from under nutrition, which leads to impaired iron absorption, and this under nutrition is attributed to a deficiency in the consumption of iron-rich foods (70). The findings of our research align with the studies conducted by Kube OT et al. in 2016 in Kenya (71), as well as investigations in Bahrain (72) and among pregnant women in Ghana (73). Additionally, a study by Annan RA et al. in 2021 in the Ashanti Region, Ghana indicated an association between MUAC and the prevalence of iron deficiency anaemia. Specifically, individuals with reduced MUAC measurements were more predisposed to developing IDA [27].

There is a significant association between IDA and dietary diversity scores and frequency of meals per day. Pregnant women with inadequate dietary diversity scores demonstrated 9.13 times more likely to develop IDA in contrast to those with adequate dietary diversity scores. Furthermore, those who consumed fewer than three meals daily were 4.98 folds more probable to develop IDA in contrast to had three or more meals.

The physiological demands of pregnancy necessitate a significantly elevated nutritional intake, as the nutritional requirements increase substantially to support both the pregnant individual and the developing fetus. A common issue observed is the inadequate consumption of dietary iron, leading to insufficient iron levels in the foods consumed. As a result, there is a decline in overall iron levels, and these lead to developing IDA. This conclusion corresponds with the outcomes of investigations performed in jigjiga, Somali region and Northern Ghana (74, 75).

A research investigation carried out in Kenya revealed a noteworthy finding positive linear correlation between dietary diversity and maternal IDA (76). In a study by Laelago *et al*. in 2023 in the Wolayta Zone of Southern Ethiopia, it was found that pregnant women with an inadequate dietary diversity score were 5.2 folds more likelihood to develop IDA opposed to those with an adequate score (48). Additionally, a study by Diana *et al*. in 2019 in Indonesia highlighted that a less diversified diet, particularly one deficient in dietary iron, was a contributing factor to IDA among pregnant women (77). Similarly, an investigation executed by Annan RA *et al*, 2021 among the expectants in Shanti Region of Ghana found that inadequate DDS were more prone to depleted body iron stores and the development of IDA compared to their counterparts with adequate scores (78).

Numerous studies indicate that the diverse dietary habits of pregnant women can significantly affect their iron levels, potentially resulting in IDA during pregnancy. A study conducted in Westmoreland, Jamaica, (79) along with a study by Burayu ET and Degefa BD, (80) in southwestern Ethiopia, as well as findings from Thailand, (81) demonstrate that a pregnant women who include green leafy vegetables in their diets develop a lower risk of developing IDA in contrast to don’t consume such edible plants.

This indicates that consuming a variety of diets may enhance iron levels, thereby decreasing the risk of developing IDA. It is essential to continue educating pregnant women on the significance of incorporating more iron-rich foods into their diets while minimizing or limiting the intake of foods that hinder iron absorption.

7. Strength and limitation of the study

- The efficacy of this research is evident among pregnant women, with a pronounced focus on dietary assessment, as the dietary intake is directly associated with iron storage levels.
- The research was constrained by the use of a relatively small sample size.

8. Conclusion and recommendation

8.1. Conclusion

In conclusion, our investigation found a low prevalence of iron deficiency anemia, associated with low mid-upper arm circumference, dietary diversity scores under five, and lower meal frequency. Pregnant women with a MUAC of less than 23 cm, inadequate dietary diversity scores and consumed meals fewer than three times daily were found to have an increased risk of developing iron deficiency anaemia compared to their counterparts.

8.2. Recommendations

- It is essential to provide health education to pregnant mothers regarding the significance of the vital components of maternal nutrition, with a particular emphasis on dietary diversity.
- Community-based research is essential to explore the factors influencing iron deficiency anemia in pregnant women. Such studies should include larger sample sizes and adopt a longitudinal design, as cross-sectional approaches are insufficient for establishing strong causal relationships.

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ANNEX I

Information Sheet

Title of the research project: - Magnitude and Determinants of Iron Deficiency Anemia among First Antenatal Care Attending Pregnant Women at Selam Health Center, Addis Ababa, Ethiopia.

Name of the organization: - Addis Ababa University, Department of Medical laboratory sciences

Purpose of the study: - The purpose of this study is to provide necessary information about the IDA and its socio-demographic distribution among the first ANC pregnant women attending at Selam Health Center, Addis Ababa, Ethiopia.

Study Participation Procedures: - To participate in this study, you'll sign a consent form first. Then, we'll gather socio-demographic data, risk factors, fasting blood, and stool samples using a questionnaire. Thanks for your cooperation!

Benefits and Risks of the Study: - The study will provide the prevalence of IDA in first ANC attendees at Selam Health Center, Addis Ababa. Sample collection may cause discomfort, and questionnaires might lead to mild stress, but experienced professionals will ensure your safety.

Confidentiality: - Your information will remain confidential, with no identifiers used only code numbers. Specimens will be used solely for this study.

Voluntary participation: - Participation in this study is voluntary, and you may withdraw at any time. Ethical approval will be obtained from the Addis Ababa University Research Ethical Review Committee. Feel free to ask questions now or in the future if needed.

Here are addresses of principal investigator and advisors who you can contact:

		Mobile phone	**e-mail**
Name of investigator	Kedir Dendir	+251922995332	kedirdendir20@gmail.com
Name of advisors	Dr. Zemenu Tamir	+251 915992362	zemenut266@gmail.com
	Mr. Moges Wordofa	+251984742173	heranmakmow@gmail.com
DRERC		**+2512755170**	

ANNEX II

Consent Form

Participant identification number ________________________

Full name of participant________________________________

I have read the information sheet fully or have been read to me fully in my own language the information on it. I understood that the research project has got ethical approval from the Research Ethical Review Committee of the College of Health Science, Addis Ababa University. And also, I understood well the information about the purpose and benefits of the study, procedures to be carried out, discomforts and risks during sampling as well the confidentiality of the information and other information regarding the study. Therefore, I am volunteer to participate obtain the specimen and give information.

I______________________________ have given my consent freely for the participation on the study on prevalence.

Signature _________________, Date________________________

Data collector name: __________________

Signature: _____________________

Date: _________________________

ANNEX III

Questionnaire English version

Addis Ababa University College of Health Science Department of Medical Laboratory Science

Title: Magnitude and Determinants of Iron Deficiency Anemia among First Antenatal Care AttendingPregnant Women at Selam Health Center, Addis Ababa, Ethiopia.

Part I: Socio demographic characteristics of pregnant women

NO_	Variables	Responses	
1.	Age	________________	
2.	Occupation	A) Governmental employee B) Private employees C) Merchant D) House wife E) Daily labor F) Student	
3.	Education	A)Informal	B) Formal
4.	Residence	A) Rural	B) Urban
5.	Your family size?	A) Less than 5	B) 5 and more

Part II: **Diet assessment**

6	Have you ever heard dietary information with respect to pregnantwoman?	A) Yes B) No
7	How many times meals do youconsume per day?	A) One times B) Two times C) Three times D) More than three times
8	Do you have any food allergies?	A) Yes B) No

9	Do you take any medications or have any medical conditions that affect your eating habits?	A) Yes B) No
10	Have you experienced any dental problems that affect your eating habits?	A) Yes B) No

Minimum dietary diversity: women (MDD-W) check list

Food Types		**Score**	
		0	**1**
11	Grains, white roots, tubers, and plantains		
12	Pulses (beans, peas, lentils)		
13	Nuts and seeds		
14	Dairy products (milk, yogurt, cheese)		
15	Meat, poultry, fish, and seafood		
16	Eggs		
17	Dark green leafy vegetables		
18	Other vitamin-A rich fruits and vegetables (such as carrots, mangoes, etc.)		
19	Other vegetables (not vitamin-A rich)		
20	Fruits (other than those rich in vitamin A)		
Sum			

Part III: clinical and gynecological characteristics

21	Have you information about amenia?	A Yes B No
22	Have you a child before?	A) Yes B) No
23	How many children do you have?	______________

24	Do you have any history of abortion with in last year?	A) Yes B) No
25	How often you suffer in morning sickness?	A) Never B) Rarely C) Sometimes D) Always
26	Gravidity	________________
27	Trimester	________________
28	MUAC	____________________cm

አባሪ I

የመረጃ ወረቀት

የምርምር ፕሮጀክቱ ርዕስ፡- በአዲስ አበባ በሰላም ጤና ጣቢያ የደም ማነስ ምክንያት የሆነው በሰውነት የብረት እጥረት ማነስ በቅድመ ወሊድ እርጉዝ ሴቶች ላይ ያለበትን ሁኔታ ማየት።
የድርጅቱ ስም ፡ - አዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል

የጥናቱ አላማ፡- የዚህ ጥናት አላማ ስለ ደም ማነስ አስፈላጊ መረጃዎችን ለመስጠት እና በማህበረሰቡ ያለው ስርጭት ለማወቅ ሲሆን በመረጃዎቹም የነፍሰ ጡር እናቶች ክትትል በሰላም ጤና ጣቢያ ምን እንደ ሚመስል ያሳያል።

የጥናቱ ጥቅሞች ፡ - ግኝቱ በመጀመሪያ ለነፍሰ ጡር እናቶች በሰላም ጤና ጣቢያ ውስጥ ስለ በሽታው ስርጭት እና ምንነት መረጃ ይሰጣል በመቀጠልም ለማህበረሰቡ ስለ ደም ማነስ ግንዛቤ ይፈጥራል።

ሚስጥራዊነት: - ስለእርስዎ የተገኘ ማንኛውም መረጃ በሚስጥር ይጠበቃል። ይህ ስለእርስዎ ማንኛውንም መለያ መጠቀምን በማስወገድ የሚያረጋግጥ ነው እና መረጃ በኮድ ቁጥር ይመዘገባል። የእርስዎ ናሙና ለዚህ ጥናት ዓላማ ብቻ ጥቅም ላይ ይውላል።

በፈቃደኝነት ተሳትፎ ፡ - በዚህ ጥናት ላይ መሳተፍ በፈቃደኝነት ላይ የተመሰረተ ሲሆን በማንኛውም ጊዜ ለመሳተፍ እምቢ የማለት መብት አለዎት. የሥነ ምግባር ክሊራንስ ተገኝቶ በአዲስ አበባ ዩኒቨርሲቲ የምርምር የሥነ ምግባር ገምጋሚ ኮሚቴ እንደፀደቀ ለማሳወቅ እወዳለሁ። በመጨረሻም፣ እየተሰራ ያለ ነገር ካልተረዳሽ አሁንም እና ወደፊት ጥያቄዎችን መጠየቅ ይቻላል። ልታገኛቸው የምትችላቸው የዋና መርማሪ እና አማካሪዎች አድራሻ እነኚህ ናቸው፡

የመርማሪው ስም፡- ከድር ደንድር ሞባይል፡ 0922995332
የአማካሪዎች ስም: - ዶ/ር ዘመኑ ታምር ፡ - ሞባይል +251915992362
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የአዲስ አበባ ዩኒቨርሲቲ የህክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የሪሰርችና ስነ-ምግባር ኮሚቴ
አድራሻ ፡ ስልክ፡- +2512755170

አባሪ II

ፍቃድ ቅፅ

የተሳታፊ መለያ ቁጥር _______

የተሳታፊ ሙሉ ስም _________________________

የመረጃ ወረቀቱን ሙሉ በሙሉ አንብቤአለሁ ወይም ሙሉ በሙሉ በራሴ ቋንቋ አዳምጫለሁ:: ከመረጃ ወረቀት ላይ የምርምር ፕሮጀክቱ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የምርምር የሥነ ምግባር ግምገማ ኮሚቴ የስነምግባር ማረጋገጫ እንዳገኘ ተረድቻለሁ:: እንዲሁም የመረጃው ዓላማ እና ጥቅሞቹ፣ በናሙና ወቅት የሚከናወኑ ሂደቶች፣ እና አደጋዎች እንዲሁም የመረጃ ምስጢራዊነት እና ሌላ መረጃ በተመለከተ ከመረጃ ወረቀት ላይ ተረድቻለሁ:: ስለዚህም እኔ ለመሳተፍ ፈቃደኛ በመሆኔ ናሙና እና አስፈላጊውን መረጃ እሰጣለሁ::

ፊርማ ______ ___________________, ቀን________________________________

መረጃ ሰብሳቢ ስም: ___________________________

ፊርማ: _________________________________

ቀን:- ____ _________________________________

አባሪ III

መጠይቅ

አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል

በ አዲስ አበባ በሰላም ጤና ጣቢያ የደም ማነስ ምክንያት የሆነው በሰውነት የብረት እጥረት ማነስ በቅድመ ወሊድ እርጉዝ ሴቶች ላይ ያለበትን ሁኔታ ማየት

የታካሚ ካርድ ቁጥር_______________________________ የቃለ መጠይቅ ቀን_______________

ክፍል 1፡ የስነ ሕዝብ ባህሪያት

ተ.ቁ	ተለዋዋጮች	ምላሾች
1.	ዕድሜ	_______________________________
2.	ሥራ	A) የመንግሥት ሰራተኛ B) የግል ስራ C) ነጋዴ D) የቤት እመቤት E) ጉልበት ሥራ F) ተማሪ
3.	የትምህርት ደረጃ	A) ኢ-መደበኛ B) መደበኛ
4.	መኖሪያ	ሀ. ገጠር ለ. ከተማ
5.	የቤተሰብ መጠን?	A) ከ 5 በታች B) 5 እና ከዛ በላይ

ክፍል 2፡ የአመጋገብ ግምገማን በተመለከተ

6.	የእርጉዝ ሴት አመጋገብ መረጃ አለሽ?	A) አዎ B) አይ
7.	በቀን ውስጥ ምን ያህል ጊዜ ትመገቢያለሽ?	A) አንድ ጊዜ B) ሁለት ጊዜ C) ሶስት ጊዜ D) ከሶስት ጊዜ በላይ
8.	የምግብ አለርጂ አለብሽ?	A) አዎ B) አይ
9.	አመጋገብሽ ላይ ተፅዕኖ የሚያመጣ ችግር ወይም የምትወስጅው መድሀኒት አለ?	A) አዎ B) አይ

10.	ምግብ ለመመገብ የሚያስቸግር የጥርስ ህመም አለሽ?	A) አዎ B) አይ

የእርጉዝ ሴት የአመጋገብ ሁኔታን የሚያሳይ መጠይቅ			
የምግብ ዓይነቶች		ነጥብ	
		0	1
11	ጥራጥሬዎች, ነጭ ሥር ያላቸው ምግቦች		
12	ጥራጥሬዎች (ባቄላ, አተር, ምስር)		
13	ፍራፍሬዎች እና ዘሮች		
14	የወተት ተዋጽኦዎች (ወተት፣ እርጎ፣ አይብ)		
15	ስጋ, የዶሮ ሰጋ, አሳ እና የባህር ምግቦች		
16	እንቁላል		
17	ጥቁር አረንጓዴ ቅጠላማ አትክልቶች		
18	ሌሎች በቫይታሚን ኤ የበለጸጉ ፍራፍሬዎችና አትክልቶች (እንደ ካሮት፣ ማንጎ፣ ወዘተ.)		
19	ሌሎች አትክልቶች (በቫይታሚን ኤ ከበለፀጉ ውጭ)		
20	ፍራፍሬዎች (በቫይታሚን ኤ ከበለፀጉ ውጭ)		
ድምር			

ክፍል 3፡ የህክምና እና ሰነ-ተዋልዶን በተመለከተ

21	ስለ ደም ማነስ መረጃ አለሽ?	A) አዎ B) አይ
22	ከዚህ በፊት ልጅ አለሽ?	A) አዎ B) አይ
23	ስንት ልጆች አሉሽ?	-----------------------------
24	ባለፈው አንድ አመት ውስጥ የፅንስ ውርጃ አጋጥሞሽ ያውቃል?	A) አዎ B) አይ

25	ምን ያህል ጊዜ ቅሪት ትፋት ያጋጥምሻል?	A) በጭራሽ B) አልፎ አልፎ C) አንዳንዴ D) ሁሌም
26	የእርግዝና ብዛት	____________________
27	የእርግዝና ወር	____________________
28	MUAC(መካከለኛ የላይኛው ክንድ መጠን)	____________________ ሴ.ሜ

ANNEX IV

Laboratory standard operating procedure (SOP)

1. Hemoglobin test principle

HGB is determined by the colorimetric method. The WBC/HGB dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is measurable at 525 nm. An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then is measured by a photo-sensor that is mounted on the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading (readings taken when there is only diluent in the bath). The HGB is calculated and the final result is expressed in g/L (Mindray BC 3000 Plus Standard Operating Procedure).

2. Serum ferritin

Sandwich principle

1st incubation: 6 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complex form a sandwich complex. 2nd incubation: After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a photomultiplier (Cobas-e 411 SOP).

3. CRP test principle

C-reactive protein (CRP) is measured using latex-enhanced agglutination method. The basic principle of particle-enhanced assays is the interaction of a soluble analyte with the matching antigen or antibody attached to polystyrene particles. Particles with a polystyrene core and a hydrophilic shell are used to covalently link anti-CRP antibodies in order to quantify CRP. Latex particles coated with mouse monoclonal anti-CRP antibodies are combined with a diluted solution of the test sample. The test sample's CRP will combine with the latex particles to form

an antigen-antibody complex.

4. Stool examination test principle

Stool sample is analyzed for color, consistency, quantity, form, odor, and mucus presence macroscopically. Saline wet mount examination: The stool is emulsified in normal saline and a large drop is placed on a glass slide and is then covered with a cover slide. Then examined under a light microscope, it is important to examine specimen under 10X objective lens at first to observe large molecules, cells, ova and helminthes, then to the 40X objective to complete the test. It is preferable to keep the condenser down and the intensity of the light low for proper visualization of the ova and cysts.

DECLARATION

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

M.Sc. candidate: **Kedir Dendir**

Signature: ____________________

Date: ____________/25________

This thesis has been submitted with our approval as advisors.

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Date: ____________/25________

Place: Addis Ababa, Ethiopia.

Advisor: **Mr. Moges Wordofa**

Signature: ____________________

Date: ____________/25________

Place: Addis Ababa, Ethiopia.