

Research Article

Iron Demand and Regulation in Early Pregnancy: A Study of Erythropoietin, Hepcidin, and Iron Status at First Booking

Esther E. Odungide ¹, Loveth A. Emokpae ², Orobosa Aikoriogie ³, Grace E. Obasuyi ², Elizabeth M. Babatunde ², Mathias A. Emokpae ^{2,*}

1. St. Luke Hospital, Uyo, Akwa Ibom State, Nigeria; E-Mail: eeodungide@gmail.com
2. Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria; E-Mails: loveth.emokpae@uniben.edu; obasuyi.eleojo@uniben.edu; babatundemoyinoluwa@yahoo.com; mathias.emokpae@uniben.edu; ORCID: 0009-0009-6163-2603; 0009-0000-2542-7105; 0009-0001-9511-4224; 0000-0002-6266-1774
3. Department of Chemical Pathology, School of Medicine, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria; E-Mail: orobosa.aikoriogie@uniben.edu

* **Correspondence:** Mathias A. Emokpae; E-Mail: mathias.emokpae@uniben.edu; ORCID: 0000-0002-6266-1774

Academic Editor: Mauro Fisberg

Special Issue: [Infant and Young Child Feeding, Pregnancy Diet and Health](#)

Recent Progress in Nutrition
2026, volume 6, issue 2
doi:10.21926/rpn.2602011

Received: January 21, 2026
Accepted: June 14, 2026
Published: June 24, 2026

Abstract

During pregnancy, the increased demand for elemental iron makes pregnant women particularly susceptible to iron deficiency anemia. The management of iron homeostasis and erythropoiesis is controlled by hepcidin and erythropoietin. A comprehensive assessment that incorporates the stimulus, the regulatory gatekeeper (hepcidin), and the resulting iron status provides a robust, mechanistic framework for comprehending the initial disruptions in iron metabolism during pregnancy. This study aims to evaluate alterations in iron metabolism and erythropoietic activity among pregnant women by assessing serum erythropoietin, hepcidin, and iron status indices, and to determine the influence of gestational age at antenatal



© 2026 by the author. This is an open access article distributed under the conditions of the [Creative Commons by Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium or format, provided the original work is correctly cited.

registration and anaemia status on these parameters. Serum erythropoietin (EPO), urinary hepcidin (Hep), serum iron parameters, and complete blood count were evaluated in 200 pregnant women at their initial antenatal booking and in 150 non-pregnant women of reproductive age using standard methodologies. A total of thirty-eight (19.0%) of the pregnant women registered at ≤ 12 weeks of gestation, while 162 (81.0%) registered after 12 weeks of gestation. The average serum EPO and total iron-binding capacity were significantly elevated ($p < 0.05$). In contrast, Hep, ferritin, iron, and transferrin percentage saturation were notably decreased ($p < 0.05$) in pregnant women compared with their non-pregnant counterparts. Serum iron and ferritin levels in pregnant women who registered at ≤ 12 weeks of pregnancy were significantly higher ($p < 0.05$). In contrast, Hep, total iron-binding capacity, and percentage saturation transferrin were considerably lower ($p < 0.05$) in women who registered for antenatal care at ≤ 12 weeks compared to those who registered at > 12 weeks of pregnancy. The average serum EPO and Hep levels in anaemic pregnant women were significantly higher ($p < 0.05$) than those in non-anaemic pregnant women. However, serum ferritin, iron, total iron-binding capacity, and percentage saturation transferrin were significantly lower ($p < 0.05$) in anaemic pregnant women compared to their non-anaemic counterparts. These findings indicate that pregnancy is associated with marked alterations in iron metabolism, characterized by increased erythropoietic activity and reduced iron stores. The significantly elevated serum erythropoietin and total iron-binding capacity, alongside decreased ferritin, serum iron, hepcidin, and transferrin saturation, suggest increased iron demand and relative iron deficiency among pregnant women. Early antenatal registration (≤ 12 weeks) appears beneficial, as it is associated with better iron status and more favorable regulatory profiles compared to late registration. Overall, the results underscore the importance of early antenatal care and timely monitoring of iron status to prevent and manage iron deficiency and anaemia during pregnancy.

Keywords

Female; pregnancy; hepcidin; erythropoietin; biomarkers; iron

1. Introduction

Pregnancy is a significant physiological condition marked by substantial increases in metabolic and hematologic demands. To facilitate fetal growth and placental function, maternal blood volume increases by roughly 50%, necessitating a corresponding rise in red blood cell mass and, as a result, a considerable uptick in iron utilization [1-3]. This elevated requirement, estimated at 1000-1200 mg of elemental iron throughout gestation, makes pregnant women particularly susceptible to iron deficiency (ID) and its advancement to iron deficiency anemia (IDA). IDA during pregnancy poses a significant global health issue, linked to serious adverse effects such as maternal fatigue, heightened risk of preterm delivery, low birth weight, and hindered cognitive development in children [4, 5].

The current clinical approach to evaluating iron status during pregnancy, especially at the vital first antenatal booking visit, relies heavily on traditional hematological indicators such as hemoglobin (Hb), serum ferritin, and transferrin saturation. Although essential, these markers

exhibit considerable limitations in the fluctuating context of early pregnancy. Hemoglobin serves as a late indicator, decreasing only after iron reserves are depleted. While haemoglobin serves as an indicator of existing anaemia, ferritin detects early-stage iron deficiency (depleted iron stores), enabling timely intervention to avert severe anaemia and its associated complications during pregnancy [6, 7]. Serum ferritin, the main marker of iron reserves, acts as an acute-phase reactant that may be elevated due to the low-grade inflammation typical in pregnancy, potentially obscuring genuine iron deficiency [6-8]. This diagnostic uncertainty can postpone timely intervention.

Iron homeostasis and erythropoiesis are controlled by two important hormones: hepcidin and erythropoietin (EPO). Hepcidin, recognized as the primary regulator of systemic iron availability, regulates dietary iron absorption and the recycling of iron by macrophages through the degradation of the cellular iron exporter ferroportin [9, 10]. Inflammatory signals increase hepcidin levels, which sequester iron and contribute to the phenomenon known as “anemia of inflammation”, but iron deficiency and heightened erythropoietic demand generally lead to reduced hepcidin production [11, 12]. Importantly, urinary hepcidin has been identified as a reliable, non-invasive indicator of systemic hepcidin activity [13]. Erythropoietin (EPO) is a primary stimulator of red blood cell production, secreted by the kidneys in response to tissue hypoxia. During the early stages of pregnancy, EPO levels increase to facilitate the necessary expansion of red cell mass, generating a legitimate signal for iron demand that, under normal physiological conditions, should be accompanied by a corresponding suppression of hepcidin to release iron [13, 14].

Nevertheless, the exact relationship between the demand signal (EPO) and the regulatory signal (hepcidin) during the critical first trimester remains inadequately defined. It is postulated that early subclinical inflammation or other pregnancy-related adaptations may impair the appropriate hepcidin response to increasing EPO levels, resulting in functional iron deficiency even before any changes in standard parameters. Consequently, a comprehensive assessment that integrates the stimulus (EPO), the regulatory gatekeeper (urinary hepcidin), and the resulting iron status (ferritin, Hb, etc.) provides a robust, mechanistic framework for comprehending the earliest disruptions in iron metabolism during pregnancy.

To this point, only a few studies have simultaneously assessed serum EPO and urinary hepcidin in pregnant women [15, 16], and none have specifically examined this triad of biomarkers at the crucial first antenatal booking. This indicates a notable gap in understanding, as this moment provides the earliest chance for predictive evaluation and targeted intervention. This cross-sectional study aims to evaluate alterations in iron metabolism and erythropoietic activity among pregnant women by assessing serum erythropoietin, hepcidin, and iron status indices, and to determine the influence of gestational age at antenatal registration and anaemia status on these parameters.

2. Materials and Methods

2.1 Study Area/Population

The participants enrolled in this study are pregnant women who reported for their first antenatal booking in Edi International Hospital and Stella Obasanjo Women and Children’s Hospital, both in Benin City, Edo State, Nigeria. Stella Obasanjo Hospital is a secondary healthcare facility equipped with modern medical technologies. It is a 250-bed hospital that serves as a leading center of medical excellence, promoting innovation and delivering high-quality healthcare services. Meanwhile, Edi International Hospital operates as a private 25-bed secondary health facility offering round-the-

clock care. Its specialized services span pediatrics, obstetrics and gynecology, orthopedics, ophthalmology, and dentistry. With over five decades of experience, it prioritizes patient-centered care to ensure a comprehensive healthcare experience.

A total of 350 subjects were consecutively recruited; 200 pregnant women who registered for antenatal care for the first time, and 150 non-pregnant women (controls) who gave informed consent were enrolled. Age-matched non-pregnant women (controls) were consecutively enrolled from the staff, students, and patients who visited the hospitals for routine medical checks.

2.2 Sample Size Determination

Sample size was determined using the sample size determination formula [17] and a 16% anemia prevalence in pregnancy [18].

$$n = Z^2 P(1 - P)/d^2$$

Where:

n = minimum sample size

Z = Standard normal deviate that corresponds to 95% confidence limit, i.e. 1.96

d = alpha level of significance is 5%, i.e. 0.05

P = Prevalence rate of anaemia in pregnancy is 16%, i.e. 0.16

Therefore:

$$(1 - P) = (1 - 0.16) = 0.84$$

$$n = (1.96)^2 0.16(0.84)/(0.05)^2 = 207$$

A total of 207 pregnant women at first antenatal booking and 150 non-pregnant women were recruited for the study. However, 7 specimens from the pregnant women were lost during the investigation; data from 200 pregnant women were analyzed.

2.3 Inclusion Criteria

Pregnant women who had not commenced routine antenatal medications and who do not have sickle cell disease, as well as those who tested negative for Human Immunodeficiency Virus (HIV), Hepatitis B and C, and Syphilis, aged between 20 and 40 years, were included in the study. Age-matched non-pregnant women without sickle cell disease and who also tested negative for HIV, Hepatitis B and C, and Syphilis served as the control group.

2.4 Exclusion Criteria

Women, both pregnant and non-pregnant, with a known history of sickle cell disease, those who are HIV positive, or who have Hepatitis B and C, Syphilis, or any other chronic illnesses, as well as individuals under 18 years of age, were excluded from the study.

2.5 Ethical Considerations

Ethical approval was secured from the Ethics and Research Committee of the Ministry of Health, Edo State, with Reference number HA-577/206 dated 7th February 2020. Participants provided

informed consent, and their socio-demographic and medical histories were collected using a questionnaire.

2.6 Clinical and Anthropometric Measurements

These measurements were conducted with the assistance of qualified nurses utilizing standard scales for height and weight. Additional factors considered included age, duration of pregnancy, socioeconomic status, and previous pregnancy history, which were gathered through questionnaires completed by the participants.

2.6.1 Definition

According to the World Health Organization, the minimum acceptable hemoglobin (Hb) level during pregnancy is 11 g/dL, while for non-pregnant women, it is less than 12 g/dL [19]. The severity of anaemia in developing countries is classified as mild (10-10.9 g/dL), moderate (7-9.9 g/dL), and severe (<7.0 g/dL). However, in sub-Saharan Africa, Lawson and Harrison indicated that significant harm to the fetus does not occur until the haemoglobin level falls below 10 g/dL or the packed cell volume (haematocrit) drops to 30% [20]. Therefore, anaemia was defined as a hemoglobin level of less than 10 g/dL.

2.7 Sample Collection

A 5 mL sample was obtained from the subject for the assessment of erythropoietin, iron, full blood count, and erythrocyte sedimentation rate through venipuncture. Precisely 3 mL was transferred into an anticoagulated container at a ratio of 1.5 mg EDTA per mL of blood, designated for the full blood count and erythrocyte sedimentation rate assays. The remaining 2 mL was placed into a plain container. The samples were allowed to clot adequately and retract at room temperature within 2 hours of collection, then centrifuged at 3000 rpm for 10 minutes to separate the serum. Urine bottles were provided to subjects to collect random urine samples on the same date as blood collection. Serum and urine were aliquoted into properly labeled, clean, plain tubes and stored at -20°C in preparation for the analyses of erythropoietin, iron, and hepcidin.

2.8 Laboratory Analyses

Erythropoietin and urinary hepcidin levels were measured using Enzyme-Linked Immunoabsorbent (ELISA) kits sourced from Sunlong Biotech Co., Ltd, Zhejiang, China. Serum ferritin and iron concentrations were evaluated using the spectrophotometric method with kits acquired from ALPCO Diagnostics, Salem, NH, USA, and Tecco Diagnostics, Anaheim, CA, USA. The full blood count was performed using the SYSMEX hematology analyzer from Guangzhou, China. The erythrocyte sedimentation rate (ESR) was assessed utilizing the Westergren technique.

2.9 Calculation of the Percentage (%) Saturation of Transferrin

The percentage (%) saturation of transferrin with iron was calculated by dividing the serum iron concentration by the total iron-binding capacity (TIBC) and then multiplying the result by 100.

2.10 Statistical Analysis

The statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences) software, version 21.0. The values obtained from the study were presented as mean \pm standard deviation and were compared using the independent Student's t-test. The Pearson correlation coefficient was used to assess the relationship between variables and anemia, while the sensitivity and specificity of the various parameters were calculated as predictors of anemia during pregnancy (the cutoff endpoint is anemia according to the Lawson Criteria: less than 10 g/dL). The significance level was established at $p < 0.05$.

2.11 Ethical Issue

Ethical approval was secured from the Ethics and Research Committee of the Ministry of Health, Edo State, with Reference number HA-577/206 dated 7th February 2020. Participants provided informed consent, and their socio-demographic and medical histories were collected using a questionnaire.

3. Results

Figure 1 shows the flow of participants through the study from the beginning to the end. A total of 350 women, consisting of 200 pregnant women at various stages of pregnancy at first antenatal booking, and 150 non-pregnant women (controls), were investigated in this study. There was no significant difference in mean age between pregnant women and non-pregnant women. Weight and BMI were significantly higher ($p < 0.001$) among pregnant women than in controls. There was no statistical difference in the educational status of cases and controls. The anaemic status of the pregnant women according to the WHO anemia classification showed that 132 (66.0%) of the pregnant women were anaemic while 68 (34.0%) were non-anaemic, while in the non-pregnant women, 40 (26.7%) were anemic as against 110 (73.3%) which were non-anaemic but using Lawson Anemia classification, 120 (60.0%) of the pregnant women were anaemic while 80 (40.0%) were non-anaemic, similarly, in the non-pregnant women, 29 (19.3%) were anaemic as against 121 (80.7%) which were non-anaemic. Of the 200 pregnant women, 38 (19.0%) registered at ≤ 12 weeks of gestation, while the majority, 162 (81.0%), registered at > 12 weeks of gestation, as shown in Table 1.

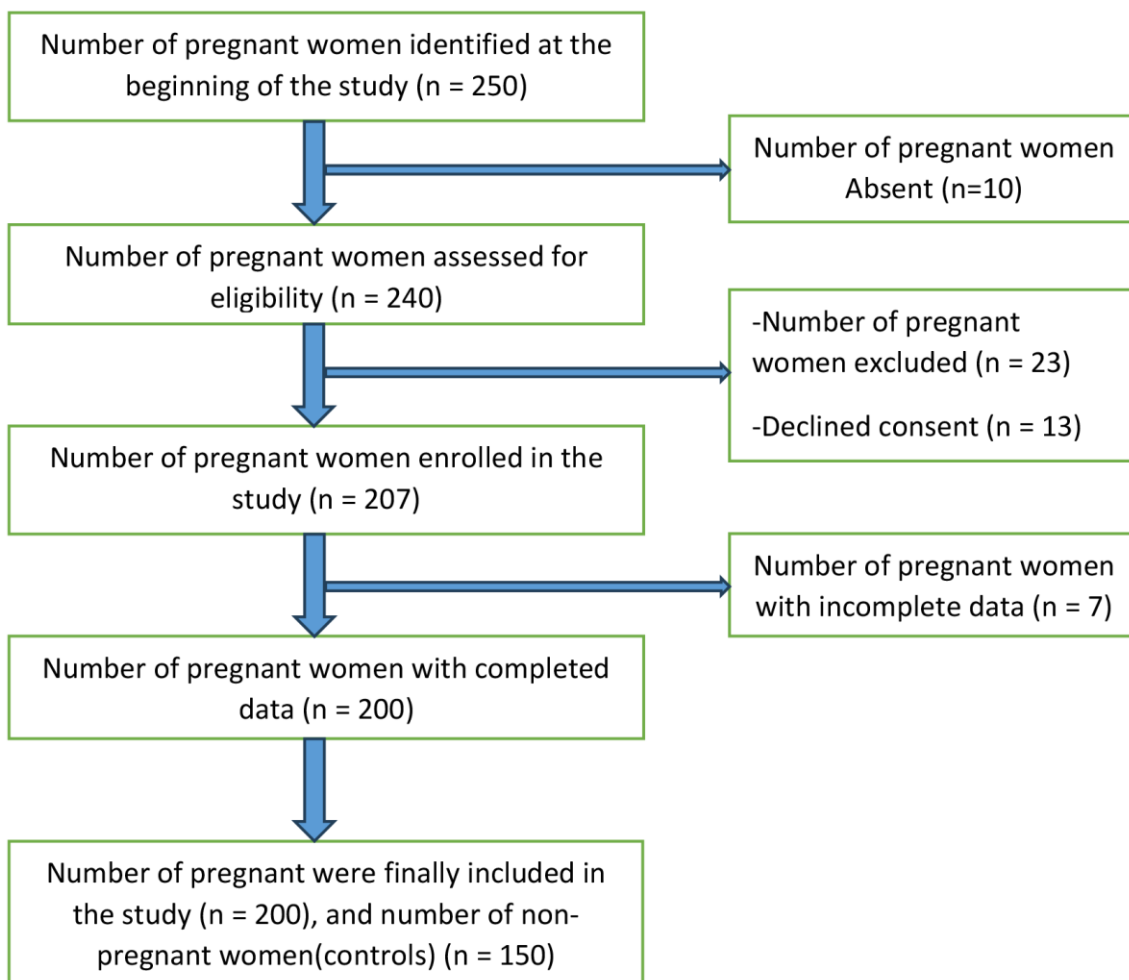


Figure 1 Consort Diagram (Flow diagram) indicating enrollment of participants in the study.

Table 1 Demographics of the studied pregnant and non-pregnant women.

Parameters	Pregnant Women n = 200 (%)	Non-Pregnant Women n = 150 (%)	p-value
Mean Age (years)	29.60 ± 0.30	29.96 ± 0.33	0.424
Mean Height (meters)	1.81 ± 0.005	1.82 ± 0.006	0.184
Mean Weight (kg)	77.20 ± 0.38	72.61 ± 0.52	0.0001
Mean BMI	23.72 ± 0.19	22.02 ± 0.21	0.0001
Mean Pregnancy Duration (Weeks)	14.24 ± 0.14	NA	NA
Occupation			
Civil Servant	62 (31.0)	40 (26.7)	3.08 (0.08)
Business	136 (68.0)	98 (65.3)	
Unemployed	2 (1.0)	12 (8.0)	
Education Status			
Primary	18 (9.0)	22 (14.7)	1.37 (0.17)
Secondary	65 (32.5)	54 (36.0)	
Tertiary	109 (54.5)	64 (42.7)	
None	8 (4.0)	10 (6.7)	
Anemic status (WHO criteria)			
Anaemic (<11 g/dl)	132 (66.0)	40 (26.7)	13.6 (0.001)
Non-Anemic (>11 g/dl)	68 (34.0)	110 (73.3)	
Anaemic status (Lawson criteria)			
Anemic (<10 g/dl)	120 (60.0)	29 (19.3)	10.4 (0.002)
Non-Anaemic (>10 g/dl)	80 (40.0)	121 (80.7)	
Pregnancy Gestational age at registration			
≤12 Weeks	38 (19.0)	NA	NA
>12 Weeks	162 (81.0)	NA	

n = number, % = per cent, NA = not applicable, BMI = Body mass index. $p < 0.05$ = statistically significant, $p > 0.05$ = not statistically significant, Categorical variable compared using chi-square test.

Table 2 shows the mean values of measured hematological parameters for pregnant and non-pregnant women. The mean red blood cell count, haemoglobin, haematocrit, platelet count, lymphocyte, and monocyte were significantly lower ($p < 0.05$) among pregnant women than non-pregnant women. Conversely, total white blood cell count, neutrophil, erythrocyte sedimentation rate, mean cell volume, and mean cell hemoglobin were significantly higher ($p < 0.05$) in pregnant women than in non-pregnant women.

Table 2 Comparison of Basic Hematological indices for pregnant and non-pregnant Women.

Haematological Parameters	Pregnant Women (N = 200) (Mean ± SD)	Non-Pregnant Women (N = 150) (Mean ± SD)	Student's t-test	p-value
Red Blood Cell ($\times 10^6/\mu\text{L}$)	3.99 ± 0.05	4.47 ± 0.05	-6.90	<0.001
Haemoglobin (g/dL)	10.66 ± 0.11	11.56 ± 0.13	-6.26	<0.001
Haematocrit (%)	32.99 ± 0.32	36.34 ± 0.29	-7.46	<0.001
Mean Cell Volume (μm^3)	84.44 ± 0.52	82.26 ± 0.45	3.06	<0.001
Mean Cell Haeamoglobin (pg/dL)	27.82 ± 0.50	26.56 ± 0.19	2.36	0.02
Platelet ($\times 10^3/\mu\text{L}$)	159.61 ± 3.31	172.15 ± 5.09	-2.15	0.03
Erythrocyte Sedimentation Rate (mm/hr)	65.62 ± 1.75	19.73 ± 0.53	22.19	<0.001
Total White Blood Cell Count ($\times 10^3/\mu\text{L}$)	6.27 ± 0.10	4.48 ± 0.08	13.21	<0.001
Lymphocyte (%)	33.76 ± 0.46	43.57 ± 1.08	-9.09	<0.001
Monocyte (%)	5.58 ± 0.13	6.30 ± 0.12	-3.83	<0.001
Neutrophil (%)	60.51 ± 0.52	51.25 ± 1.05	8.47	<0.001

n = number, p < 0.05 = statistically significant, p > 0.05 = not statistically significant.

Table 3 shows mean serum erythropoietin, urinary hepcidin and iron status for pregnant n and non-pregnant women. The mean serum erythropoietin (p < 0.001) and total iron binding capacity (p < 0.002) were significantly higher. In contrast, urinary hepcidin (p < 0.001), serum ferritin (p < 0.001), iron (p < 0.03), and percentage saturation transferrin (p < 0.004) were significantly lower among pregnant women than non-pregnant women.

Table 3 Concentration of Serum Erythropoietin, urinary hepcidin, and iron status parameters of pregnant and non-pregnant women.

Parameters	Pregnant Women (N = 200) (Mean ± SD)	Non-Pregnant Women (N = 150) (Mean ± SD)	Student's t-test	p-value
Serum Erythropoietin (mIU/mL)	8.88 ± 0.19	5.77 ± 0.13	12.51	<0.001
Urinary hepcidin (ng/mL)	13.54 ± 0.17	15.87 ± 0.15	-10.01	<0.001
Serum ferritin (ng/mL)	12.02 ± 1.12	52.28 ± 2.12	-24.63	<0.001
Serum Iron ($\mu\text{g}/\text{dL}$)	57.38 ± 0.37	68.04 ± 0.83	-2.07	0.03
Total Iron Binding Capacity ($\mu\text{g}/\text{dL}$)	344.75 ± 0.61	312.56 ± 0.58	4.83	0.002
% Transferrin	17.64 ± 1.41	22.79 ± 1.22	-3.96	0.004

n = number, p < 0.05 = statistically significant, p > 0.05 = not statistically significant.

The red blood cell count, haemoglobin (p > 0.001), and hematocrit were higher (p < 0.05) among pregnant women who registered at ≤ 12 weeks than those who registered at >12 weeks of pregnancy. Conversely, the mean MCHC, platelet, and erythrocyte sedimentation rate were significantly lower (p < 0.05) among pregnant women who registered ≤ 12 weeks than those who registered for

antenatal care at >12 weeks of pregnancy. The mean concentrations of mean cell volume, mean cell haemoglobin, total white blood cell count, lymphocytes, monocytes, and granulocytes were not significantly different between the groups (Table 4).

Table 4 Mean levels of measured hematological parameters of pregnant women according to gestation age of the pregnancy at which the women registered for antenatal.

Measured Hematological Parameters	Pregnancy Gestational Age		T-test	P-value	Significant level
	≤12 weeks	>12 weeks			
Red Blood Cell ($\times 10^6/\mu\text{L}$)	4.69 \pm 0.13	3.86 \pm 0.04	7.52	0.001	S
Haemoglobin (g/dL)	12.91 \pm 0.42	10.23 \pm 0.07	10.74	0.001	S
Haematocrit (%)	39.49 \pm 1.15	31.75 \pm 0.20	11.45	0.001	S
Mean Cell Volume (μm^3)	84.28 \pm 0.91	84.47 \pm 0.59	-0.13	0.89	NS
Mean Cell Haemoglobin (pg)	27.50 \pm 0.35	27.88 \pm 0.59	-0.28	0.78	NS
Mean Cell Haemoglobin Concentration (g/dL)	32.61 \pm 0.16	32.11 \pm 0.08	2.49	0.01	S
Platelet ($10^3/\mu\text{L}$)	132.94 \pm 6.83	164.69 \pm 3.59	-3.62	0.001	S
Erythrocyte Sedimentation Rate (mm/hr)	53.69 \pm 3.54	67.89 \pm 1.92	-3.05	0.003	S
Total White Blood Cell Count ($\times 10^3/\mu\text{L}$)	6.02 \pm 0.31	6.32 \pm 0.11	-1.09	0.28	NS
Lymphocyte (%)	33.37 \pm 0.97	33.83 \pm 0.52	-0.37	0.71	NS
Monocyte (%)	5.49 \pm 0.21	5.59 \pm 0.16	-0.29	0.78	NS
Neutrophil (%)	61.14 \pm 1.01	60.39 \pm 0.59	0.52	0.60	NS

n = number, $p < 0.05$ = statistically significant, $p > 0.05$ = not statistically significant, S = significant, NS = not significant.

The mean serum iron and ferritin in pregnant women who registered for antenatal care at ≤ 12 weeks of pregnancy were significantly higher ($p < 0.05$) than those who registered at > 12 weeks of pregnancy. The mean urinary hepcidin, total iron binding capacity, and percentage saturation transferrin were significantly lower ($p < 0.05$) in pregnant women who registered for antenatal at ≤ 12 weeks of pregnancy than those who registered for antenatal at > 12 weeks of pregnancy. Serum erythropoietin levels did not differ significantly between the groups, as shown in Table 5.

Table 5 Mean Serum Erythropoietin, Urinary hepcidin and iron status according to the gestational age of the pregnancy at which the women registered for antenatal.

Parameters	Pregnancy Gestational Age		T-test	P-value	Significant level
	≤12 weeks 32 (16.0%)	>12 weeks 168 (84.0%)			
Serum Erythropoietin (mIU/mL)	9.06 ± 0.43	8.84 ± 0.21	0.41	0.68	NS
Urinary hepcidin (ng/mL)	11.46 ± 0.24	13.93 ± 0.18	-5.95	0.001	S
Serum ferritin (ng/mL)	14.24 ± 0.52	10.37 ± 0.70	-6.76	0.001	S
Serum Iron (µg/dL)	58.81 ± 0.22	56.52 ± 0.52	-1.04	0.08	NS
Total Iron Binding Capacity (µg/dL)	326.05 ± 0.52	362 ± 0.92	2.83	0.04	S
% Transferrin	15.83 ± 0.92	18.46 ± 1.01	4.96	0.001	S

n = number, p < 0.05 = statistically significant, p > 0.05 = not statistically significant, S = significant, NS = not significant.

Table 6 shows the measured hematological parameters by anemic status of pregnant women according to Lawson's criteria. The red blood cell count, hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin concentration in anemic pregnant women were significantly lower (p < 0.05) compared to non-anemic pregnant women. The platelet count and erythrocyte sedimentation rate in anemic pregnant women were, however, significantly higher compared to those of non-anemic pregnant women. But the mean cell haemoglobin, total white blood cell, lymphocyte, monocyte and granulocyte in anemic pregnant women were not significantly different from those in non-anaemic pregnant women.

Table 6 Mean level of measured hematological parameters of pregnant women according to anemic status of the pregnant women.

Measured Hematological Parameter	Anemia Status (Lawson Criteria)		T-test	P-value	Significant level
	Anemic (<10 g/dL)	Non-anemic (≥10 g/dL)			
Red Blood Cell (×10 ⁶ /µL)	3.78 ± 0.14	4.40 ± 0.08	-7.24	<0.001	S
Haemoglobin (range) g/dl (11.0-14.7)	9.88 ± 0.07	12.17 ± 0.21	-12.79	<0.001	S
Haematocrit (%)	30.95 ± 0.21	36.94 ± 0.62	-11.42	<0.001	S
Mean Cell Volume (µm ³)	83.32 ± 0.71	86.61 ± 0.58	-3.06	0.002	S
Mean Cell Haemoglobin (pg)	27.61 ± 0.75	28.23 ± 0.19	-0.59	0.56	NS
Mean Cell Haemoglobin Concentration (g/dL)	31.90 ± 0.09	32.76 ± 0.11	-5.92	0.001	S
Platelet (×10 ³ /µL)	165.29 ± 4.23	148.59 ± 5.00	2.42	0.02	S
Erythrocyte Sedimentation Rate (mm/hr)	68.67 ± 2.37	59.71 ± 2.10	2.46	0.02	S
Total White Blood Cell Count (×10 ³ /µL)	6.20 ± 0.12	6.40 ± 0.19	-0.91	0.36	NS
Lymphocyte (%)	34.12 ± 0.57	33.05 ± 0.77	1.11	0.27	NS
Monocyte (%)	5.57 ± 0.18	5.61 ± 0.17	-0.13	0.89	NS
Neutrophil (%)	59.95 ± 0.65	61.60 ± 0.86	-1.51	0.13	NS

n = number, p < 0.05 = statistically significant, p > 0.05 = not statistically significant, S = significant, NS = not significant.

Table 7 indicates the comparison of the levels of serum erythropoietin, urinary hepcidin and iron status parameters between anaemic and non-anaemic pregnant women based on Lawson criteria (Hb < 10 g/dL). The mean serum erythropoietin and urinary hepcidin of anaemic pregnant women were significantly higher ($p < 0.05$) than those of non-anaemic pregnant women. Still, serum ferritin, iron, total iron binding capacity, and percentage saturation transferrin in anaemic pregnant women were significantly lower ($p < 0.05$) compared to non-anaemic pregnant women.

Table 7 Mean level of Serum Erythropoietin, Urinary hepcidin, and Iron status parameter by Anemic status.

Measured Hematological Parameter	Anemia Status (Lawson Criteria)		T-test	P-value	Significant level
	Anemic (<10 g/dL)	Non-anemic (≥ 10 g/dL)			
Serum Erythropoietin (mIU/mL)	8.38 \pm 0.22	6.74 \pm 0.18	5.79	<0.001	S
Urinary hepcidin (ng/mL)	15.37 \pm 0.19	13.73 \pm 0.16	6.65	<0.001	S
Serum ferritin (ng/mL)	24 \pm 1.08	36.84 \pm 4.34	-18.94	<0.001	S
Serum Iron (μ g/dL)	38.81 \pm 0.52	68.45 \pm 0.72	-17.04	<0.001	S
Total Iron Binding Capacity (μ g/dL)	287.76 \pm 0.82	398.03 \pm 0.99	-18.92	<0.001	S
% Transferrin	13.87 \pm 1.12	17.28 \pm 1.32	-6.96	<0.001	S

n = number, $p < 0.05$ = statistically significant, $p > 0.05$ = not statistically significant, S = significant, NS = not significant.

Table 8 shows the cut-off points, sensitivities, and specificities of the different parameters as predictors of anemia in pregnancy. Haemoglobin cut-off point is 10.95 g/dL with 100% sensitivity and 95% specificity, haematocrit cut-off was 30.45% with 95% sensitivity and 87.0% specificity, serum erythropoietin cut-off for anemia is 5.05 mIU/mL with 92% sensitivity and 62% specificity. In contrast, the cut-off, sensitivity, and specificity of urinary hepcidin for anemia detection was 11.9 ng/mL, 95%, and 73%. Also, the serum iron cut-off was 30.5 μ g/dL with 96.0% sensitivity and 100% specificity, while the serum ferritin cut-off was 18.5 ng/dL with 92% sensitivity and 100% specificity, respectively.

Table 8 The sensitivity and specificity of measured parameters.

Parameters	Cut-off	Sensitivity	Specificity
Haemoglobin (g/dL)	10.26	1.00	0.95
Haematocrit (%)	30.45	0.95	0.87
Serum Erythropoietin (mIU/mL)	5.05	0.92	0.62
Urinary hepcidin (ng/mL)	11.9	0.95	0.73
Serum Iron (μ g/dL)	30.5	0.96	1.00
Serum Ferritin (ng/mL)	18.5	0.92	1.00

4. Discussion

This research indicates that pregnancy is associated with marked changes in iron metabolism, marked by increased erythropoietic activity and reduced iron stores. The significantly elevated

serum erythropoietin and total iron-binding capacity, and decreased ferritin, serum iron, hepcidin, and transferrin saturation, suggest a state of high iron demand and relative iron deficiency among pregnant women. Early antenatal registration (≤ 12 weeks) appears beneficial, as it is associated with better iron status and more favorable regulatory profiles compared to late registration.

The study finds that a substantial majority (81%) of women scheduled for their first antenatal visit after 12 weeks of gestation do so later than the World Health Organization's recommended guidelines. This prevalent real-world trend of late-first bookings suggests that these adaptive and potentially deficient conditions may already be present before formal antenatal care commences. This finding aligns with previous research [21-23]. However, this figure exceeds the rates of 49.5% [24], 68.8% [25], and 56.98% [26] reported in Tanzania and Ethiopia. Late bookings result in pregnant women missing timely early pregnancy assessments that are crucial for favorable pregnancy outcomes. Authors have suggested that women who made late bookings were found to be twice as likely to experience anaemia compared to those who booked early. Additionally, late bookers were reported to be six times more likely to have low-birth-weight infants than their early booking counterparts [24-26].

The absence of significant differences in socioeconomic factors such as occupation and education between the cases and controls reinforces the conclusion that the observed changes in biomarkers are mainly influenced by the pregnancy itself, rather than by confounding demographic factors [27]. The haematological profile of women in the early stages of pregnancy is characterized by a unique pattern of dilutional anaemia accompanied by reactive erythropoietic characteristics, a physiological increase in leukocytes attributed to neutrophils, and mild thrombocytopenia. These alterations align with and support previously documented hormonal influences (increased EPO, suppressed hepcidin [28]). It is vital to recognize this pattern as a normal physiological response to prevent unnecessary investigations, while remaining alert to identify any values that may deviate significantly from this expected adaptive baseline.

The comparative examination of hematological parameters illustrated in Table 2 offers a detailed overview of the significant physiological adjustments the maternal hematopoietic system undergoes by the time of the first antenatal booking. The identified pattern does not suggest a pathological condition; instead, it reflects a coherent and complex response to the requirements of pregnancy.

The findings directly support the study's emphasis on biomarkers related to iron demand and regulation. They indicate that by the first antenatal booking, the maternal organism has already transitioned into a state of "regulated iron deficiency," characterized by a hepcidin-EPO axis that is actively mobilizing iron for erythropoiesis. The measurable iron reserves are being systematically depleted. This condition is notably more pronounced in women who book after 12 weeks, highlighting the dynamic aspect of this adaptation. The clinical implications are clear: reliance solely on conventional haematological indices such as haemoglobin is inadequate. Evaluating ferritin (a marker of iron stores) and understanding the altered regulatory environment (via hepcidin and EPO) are crucial for the early identification of genuine iron deficiency and the prompt initiation of treatment, potentially before overt anemia manifests. This biomarker profile offers a physiological basis for advocating early and proactive iron supplementation during pregnancy, ideally commencing in the first trimester.

The identified pattern, significantly higher serum erythropoietin (EPO) and total iron-binding capacity (TIBC) alongside significantly reduced urinary hepcidin, serum ferritin, serum iron, and

transferrin saturation, provides a coherent representation of a system that prioritizes iron mobilization for erythropoiesis at the cost of iron reserves. This triad of findings is essential for understanding the physiology of iron demand during pregnancy.

The notable suppression of urinary hepcidin signifies a vital adaptive response. Hepcidin, which regulates the entry of iron into plasma, is downregulated to enhance dietary iron absorption and promote the release of recycled iron from macrophages. This suppression directly addresses the perceived need for iron.

Simultaneously, the substantial increase in serum EPO, which catalyzes heightened red blood cell production, is driven by both the relative anaemia resulting from plasma volume expansion and the emerging iron deficiency. The alterations in both regulatory hormones (hepcidin decreasing and EPO increasing) compellingly illustrate the body's coordinated effort to satisfy the rising iron requirements of the fetus and the expanding maternal red cell mass [28, 29].

Conventional parameters of iron status validate the implications of this regulatory shift. The significantly reduced levels of serum ferritin, serum iron, and transferrin saturation indicate a swift depletion of iron reserves and a reduction in the circulating iron available for hemoglobin synthesis. The increased TIBC further indicates the body's ability to optimize iron transport capacity in a context of limited iron availability. This overall profile is indicative of developing iron-deficient erythropoiesis, which precedes and underpins the high incidence of anemia observed in this group [30, 31].

Moreover, the comparison between women booking at ≤ 12 weeks and >12 weeks of gestation (Table 4) provides a significant temporal perspective on the evolution of these changes. The observation that haemoglobin, haematocrit, and RBC count were elevated in the earlier booking group implies a less pronounced dilutional anaemia during the initial trimester [32, 33]. In contrast, the notably lower MCHC and elevated ESR observed in the later booking group suggest a progression towards more pronounced hemoglobin dilution and inflammation-related alterations as pregnancy progresses. The absence of a significant difference in MCV and MCH between these groups indicates that macrocytic compensation (potentially due to EPO stimulation or folate) is an early and persistent response. Most critically, this comparison of gestational age suggests that the essential regulatory changes (hepcidin suppression, EPO increase, and depletion of iron stores) are already initiated early in the first trimester and become more firmly established by the second trimester, underscoring a very limited window for preventive intervention. This aligns with previous reports indicating that elevated hepcidin levels in the first trimester are linked to a reduced risk of iron deficiency anaemia in late pregnancy [15].

The findings presented in Table 5 offer a crucial temporal perspective on the study of iron biomarkers, demonstrating that the physiological adaptation to the iron demands of pregnancy is a dynamic and rapidly changing process. The comparison between women booking at or before 12 weeks of gestation and those booking after 12 weeks reveals a significant transition in iron homeostasis during the first trimester, shifting from a state of relative iron repletion to one of active depletion.

The observation that serum iron and ferritin levels were significantly higher in the ≤ 12 weeks group is critical. It suggests that, on average, women in the very early stages of pregnancy still retain measurable iron reserves. This implies that the considerable demand for iron stores for fetal-placental development and maternal erythrocyte expansion has not yet completely surpassed the body's reserves or absorptive capacity at this stage [34].

In contrast, the notably reduced urinary hepcidin levels in the ≤ 12 weeks group indicate that the regulatory response initiates almost immediately. The decrease in hepcidin represents an early, proactive adjustment aimed at improving iron absorption in anticipation of future needs, even before significant depletion of stores. This initial downregulation of hepcidin likely facilitates optimal iron acquisition during the crucial period of organogenesis [35].

Furthermore, the markedly lower Total Iron-Binding Capacity (TIBC) and transferrin saturation in the earlier booking group further clarify this staged adaptation. A reduced TIBC implies that the production of transferrin, the protein responsible for iron transport, has not yet reached its maximum upregulation. The diminished saturation percentage, despite elevated serum iron levels, suggests that the circulating transport system is not yet fully activated or saturated, which aligns with a less stressed condition [34].

The most compelling finding is the lack of a significant difference in serum erythropoietin (EPO) levels between the two gestational-age groups. This suggests that the stimulus for erythropoiesis, that is, primarily tissue hypoxia resulting from dilutional anemia, may not vary substantially between these early time points. Alternatively, it may imply that the EPO response is already at its peak very early in pregnancy due to the initial expansion of plasma volume, and therefore does not show significant differences between these subgroups. This separation between EPO (which remains similar) and hepcidin (which is more significantly suppressed early on) underscores that these two regulatory hormones, while frequently coordinated, can be affected by distinct physiological signals during early pregnancy [9, 10].

The examination of hematological parameters in relation to anaemic status (Table 6) offers essential insights into the functional implications of altered iron regulation during early pregnancy, as delineated by Lawson's criteria. The observed pattern strongly supports the conclusion that the predominant anaemia in this cohort is microcytic and hypochromic, characteristic features of iron-deficient erythropoiesis. This establishes a direct connection between the hematological profile and the biomarker evidence of iron depletion [36].

The notably elevated platelet count (thrombocytosis) in anaemic women is a well-documented reactive phenomenon associated with iron-deficiency anemia, even in the absence of pregnancy. Iron deficiency can stimulate platelet production, potentially as a compensatory mechanism or due to overlapping cytokine influences on megakaryopoiesis and erythropoiesis [37].

Upon reviewing Table 7, the findings extend the conversation beyond mere iron deficiency, emphasizing a possible dysregulation of iron metabolism in anemia during early pregnancy. Increased levels of hepcidin in anaemic women serve as a significant warning sign, suggesting that the anaemia may not solely stem from insufficient dietary intake but could also involve an inflammatory factor that hinders iron utilization [38]. Consequently, evaluating hepcidin and inflammatory markers in conjunction with standard iron assessments could facilitate a more tailored and effective approach to managing anaemia in early pregnancy.

The examination of cut-off values, sensitivity, and specificity for various parameters (as shown in Table 8) shifts the focus of the study from merely describing physiological adaptations to assessing the clinical diagnostic value of these biomarkers in identifying anaemia during early pregnancy. The findings indicate a hierarchy in diagnostic efficacy, with conventional hematological indices and iron storage markers demonstrating superior performance compared to regulatory hormones when evaluated as standalone tests, thereby highlighting the beneficial role of a multi-parameter approach.

The efficacy of haemoglobin (cut-off at 10.26 g/dL), exhibiting 100% sensitivity and 95% specificity, is predictably robust, as it serves as the primary measure for defining anaemia (according to Lawson's criteria: Hb < 10 g/dL). The nearly perfect sensitivity confirms its effectiveness in accurately identifying individuals with anaemia. The 95% specificity suggests that a measurement below this threshold is a strong indicator of true anaemia, with a minimal false-positive rate.

The remarkable diagnostic efficacy of serum ferritin (cut-off 18.5 ng/mL) and serum iron (cut-off 30.5 µg/dL), both achieving 100% specificity alongside high sensitivity (92% and 96%, respectively), represents the most crucial finding from a clinical etiological standpoint.

A specificity of 100% indicates that no non-anemic woman within this cohort had a ferritin level below 18.5 ng/mL or an iron level below 30.5 µg/dL. This establishes low ferritin as an almost flawless confirmatory test for iron-deficiency anemia in this scenario. A ferritin level dropping below this threshold at the initial visit strongly indicate that depleted iron stores are likely responsible for the anaemia, warranting immediate and potentially aggressive iron therapy.

Conversely, the regulatory hormones demonstrate a different performance profile. Serum erythropoietin (EPO) shows high sensitivity (92%) but lower specificity (62%). This reflects its physiological function: it is elevated in most cases of anemia (high sensitivity). Still, it is also raised in many non-anemic pregnant women due to the physiological dilution and EPO stimulation associated with normal pregnancy, resulting in a higher number of false positives (lower specificity). Consequently, EPO serves as a sensitive marker of erythropoietic stress but cannot independently differentiate between physiological adaptation and pathological anemia.

Urinary hepcidin exhibits a balanced yet moderate profile, with a sensitivity of 95% and a specificity of 73%. The notably high sensitivity suggests that dysregulation of hepcidin is a prevalent characteristic. The specificity of 73% indicates that it is more specific than EPO, although less so than ferritin. This pattern reinforces its role as a pathophysiological marker of potential iron blockage, but it should not be regarded as a definitive diagnostic tool for iron deficiency.

5. Conclusion

The findings highlight significant changes in iron metabolism during pregnancy, marked by increased erythropoietic activity and diminished iron reserves. Elevated levels of serum erythropoietin and total iron-binding capacity, paired with reductions in ferritin, serum iron, hepcidin, and transferrin saturation, point to a state of heightened iron demand and relative deficiency in pregnant women. Early antenatal registration (within 12 weeks) is associated with improved iron status and more favorable regulatory profiles compared to late registration. Overall, the results underscore the importance of early antenatal care and timely monitoring of iron status to prevent and manage iron deficiency and anaemia during pregnancy.

Acknowledgments

The authors appreciate the contributions of all medical, nursing, and medical laboratory services Departments towards the completion of the study.

Author Contributions

Study concept and design (MAE, EEO); acquisition of data (EEO, LAE, OA); analysis and interpretation of data (MAE, GEO, EMB); drafting of the manuscript (EEO, LAE, OA, GEO, EMB); critical revision of the manuscript for important intellectual content (MAE, LAE); administrative, technical, or material support (MAE, EEO, LAE, OA, GEO, EMB); study supervision (MAE). All authors have made a significant contribution to this study and have approved the final manuscript.

Competing Interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

1. Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in hematological parameters during pregnancy. *Indian J Hematol Blood Transfus.* 2012; 28: 144-146.
2. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy: Review articles. *Cardiovasc J Afr.* 2016; 27: 89-94.
3. Gangakhedkar GR, Kulkarni AP. Physiological changes in pregnancy. *Indian J Crit Care Med.* 2021; 25: S189-S192.
4. Zhang Q, Lu XM, Zhang M, Yang CY, Lv SY, Li SF, et al. Adverse effects of iron deficiency anemia on pregnancy outcome and offspring development and intervention of three iron supplements. *Sci Rep.* 2021; 11: 1347.
5. Obeagu GU, Altraide BO, Obeagu EI. Iron deficiency anemia in pregnancy and related complications with specific insight in Rivers State, Nigeria: A narrative review. *Ann Med Surg.* 2025; 87: 3435-3444.
6. Api O, Breyman C, Çetiner M, Demir C, Ecdar T. Diagnosis and treatment of iron deficiency anemia during pregnancy and the postpartum period: Iron deficiency anemia working group consensus report. *Turk J Obstet Gynecol.* 2015; 12: 173-181.
7. Pavord S, Daru J, Prasannan N, Robinson S, Stanworth S, Girling J, et al. UK guidelines on the management of iron deficiency in pregnancy. *Br J Haematol.* 2020; 188: 819-830.
8. De Moor V, Mesens T, Soulliaert S, van der Merwe H, Vergote S, Verheecke M, et al. Iron deficiency anaemia (IDA) in pregnancy: Screening and management. *Eur J Obstet Gynecol Reprod Biol X.* 2025; 27: 100402.
9. Correnti M, Gammella E, Cairo G, Recalcati S. Iron mining for erythropoiesis. *Int J Mol Sci.* 2022; 23: 5341.
10. Kesharwani P, Dash D, Koiri RK. Deciphering the role of hepcidin in iron metabolism and anemia management. *J Trace Elem Med Biol.* 2025; 87: 127591.
11. Quintana-Castanedo L, Maseda R, Pérez-Conde I, Butta N, Monzón-Manzano E, Acuña-Butta P, et al. Interplay between iron metabolism, inflammation, and EPO-ERFE-hepcidin axis in RDEB-associated chronic anemia. *Blood Adv.* 2025; 9: 2321-2335.
12. Foucambert P, Hang JW, Malleret B. Exploring erythropoiesis: Aging through the lens of malaria and microgravity. *Trends Parasitol.* 2025; 41: 1046-1061.

13. Laller S, Patel S, Haldar D. Role of serum and urinary hepcidin in young females of reproductive age in North India. *J Lab Physicians*. 2022; 14: 175-182.
14. Tayal A, Kaur J, Sadeghi P, Maitta RW. Molecular mechanisms of iron metabolism and overload. *Biomedicines*. 2025; 13: 2067.
15. Sun P, Zhou Y, Xu S, Wang X, Li X, Li H, et al. Elevated first-trimester hepcidin level is associated with reduced risk of iron deficiency anemia in late pregnancy: A prospective cohort study. *Front Nutr*. 2023; 10: 1147114.
16. Rosson S, Pavord S. Understanding hepcidin for iron management in pregnancy. *Tranfus Med*. 2025; 35: 109-115.
17. Lwanga SK, Lemeshow S, World Health Organization. Sample size determination in health studies: A practical manual. Geneva, Switzerland: World Health Organization; 1991.
18. Ethiopian Public Health Institute (EPHI) [Ethiopia] and ICF. Ethiopia Mini Demographic and Health Survey 2019: Key Indicators. Rockville, MD: EPHI and ICF; 2019; DDI-ETH-EMDHS-2019-V01.
19. World Health Organization. Iron Deficiency, Anaemia Assessment, Prevention, and Control. A Guide for Programme Managers. Geneva, Switzerland: World Health Organization; 2001.
20. Emokpae MA, Adesina OO. Association of total antioxidant status with severity of anaemia in pregnancy in Ogun state, Nigeria. *J Med Discov*. 2018; 3: jmd17055.
21. Jinga N, Mongwenyana C, Moolla A, Maletse G, Onoya D. Reasons for late presentation for antenatal care, healthcare providers' perspective. *BMC Health Serv Res*. 2019; 19: 1016.
22. Okagua KC, Ogbondah B, Jaja I, Okagua J. Demographic correlates of gestational age at booking among antenatal clinic attendees in secondary healthcare facilities in semiurban/rural areas of Rivers state, Southern Nigeria. *Niger Health J*. 2022; 22: 305-310.
23. Izuka EO, Obiora-Izuka CE, Asimadu EE, Enebe JT, Onyeabochukwu AD, Nwagha UI. 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: 558-565.
24. Jiwani SS, Amouzou-Aguirre A, Carvajal L, Chou D, Keita Y, Moran AC, et al. Timing and number of antenatal care contacts in low and middle-income countries: Analysis in the countdown to 2030 priority countries. *J Glob Health*. 2020; 10: 010502.
25. Fagbamigbe AF, Olaseinde O, Fagbamigbe OS. Timing of first antenatal care contact, its associated factors and state-level analysis in Nigeria: A cross-sectional assessment of compliance with the WHO guidelines. *BMJ Open*. 2021; 11: e047835.
26. Kitaw TA, Haile RN. Time to first antenatal care booking and its determinants among pregnant women in Ethiopia: Survival analysis of recent evidence from EDHS 2019. *BMC Pregnancy Childbirth*. 2022; 22: 921.
27. Nicholls-Dempsey L, Badeghiesh A, Baghlaf H, Dahan MH. How does high socioeconomic status affect maternal and neonatal pregnancy outcomes? A population-based study among American women. *Eur J Obstet Gynecol Reprod Biol X*. 2023; 20: 100248.
28. Bhoopalan SV, Huang LJ, Weiss MJ. Erythropoietin regulation of red blood cell production: From bench to bedside and back. *F1000Res*. 2020; 9: F1000 Faculty Rev-1153.
29. Mégier C, Peoc'h K, Puy V, Cordier AG. Iron metabolism in normal and pathological pregnancies and fetal consequences. *Metabolites*. 2022; 12: 129.
30. Gattermann N, Muckenthaler MU, Kulozik AE, Metzgeroth G, Hastka J. The evaluation of iron deficiency and iron overload. *Dtsch Arztebl Int*. 2021; 118: 847-856.

31. Kolarš B, Mijatović Jovin V, Živanović N, Minaković I, Gvozdenović N, Dickov Kokeza I, et al. Iron deficiency and iron deficiency anemia: A comprehensive overview of established and emerging concepts. *Pharmaceuticals*. 2025; 18: 1104.
32. Al-Khaffaf A, Frattini F, Gaiardoni R, Mimiola E, Sissa C, Franchini M. Diagnosis of anemia in pregnancy. *J Lab Precis Med*. 2020; 5: 9.
33. So H, Kwon K, Jung S, Kim K. Physiological changes and trimester-specific reference intervals for complete blood count parameters in Korean pregnant women. *Medicina*. 2025; 61: 1665.
34. Mo H, Wang Z, Qu C, Liu X. The associations of maternal serum ferritin levels with hypertensive disorders of pregnancy: A longitudinal cohort study. *Front Nutr*. 2025; 12: 1639068.
35. Ginzburg Y, An X, Rivella S, Goldfarb A. Normal and dysregulated crosstalk between iron metabolism and erythropoiesis. *Elife*. 2023; 12: e90189.
36. Županić D, Višnić A, Brenčić T, Juričić G, Honović L, Štimac T. Clinical utility of calculated haematological parameters in the diagnosis of iron deficiency anaemia in pregnant women. *Biomed Rep*. 2025; 23: 188.
37. Sharma V, Bagrodia V, Modi N, Parchwani T. Marked reactive thrombocytosis in a female with iron deficiency anaemia. *BMJ Case Rep*. 2023; 16: e256738.
38. Al-Jaf DA, Hussein AL, Niranji SS. Inflammatory biomarkers and hepcidin levels in iron deficiency anemia among women of reproductive age. *Afr J Biomed Res*. 2024; 27: 5437-5441.