International Journal of Research in Medical Sciences
Igwilo AC et al. Int J Res Med Sci. 2026 Sep;14(9):3745-3752
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20263083

Original Research Article

Trimester specific changes in nutritional and iron related biomarkers among pregnant women in Calabar, Nigeria: a longitudinal study

Anastecia Chika Igwilo[1]*, Ifeyinwa Maryann Okafor, Grace I. Amilo

[1]Department of Haematology and Blood Transfusion Science, University of Calabar Teaching Hospital Calabar, Cross River State, Nigeria
[2]Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, College of Medical Sciences, University of Calabar Teaching Hospital Calabar, Cross River State, Nigeria
[3]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria
[4]Department of Biomedical Laboratory Science, School of Health Sciences, College of Medicine and Health Sciences, University of Rwanda, Kigali, Nigeria

Received: 15 March 2026
Revised: 21 May 2026
Accepted: 27 June 2026

***Correspondence:**
Dr. Anastecia Chika Igwilo,
E-mail: anneigwilo@gmail.com

ABSTRACT

Background: Nutritional deficiencies are common in pregnant women in developing countries and contribute to poor pregnancy outcomes. This longitudinal study assessed trimester-specific trends in nutritional and iron-related biomarkers in Calabar, Nigeria.
Methods: Seventy-two pregnant women (18-41 years) were enrolled in the first trimester and followed to the third trimester at the University of Calabar Teaching Hospital. Fifty age- and parity-matched non-pregnant women served as controls. Blood samples were collected at 10, 24 and 36 weeks of gestation. Total protein, albumin, iron, unsaturated iron-binding capacity (UIBC), ferritin, folate, vitamin B12 and soluble transferrin receptors (sTfR) were analysed; total iron-binding capacity (TIBC) and transferrin saturation (TS) were calculated.
Results: Albumin, folate and vitamin B12 were significantly lower in pregnant women by the second trimester compared to non-pregnant controls. Within the pregnant cohort, albumin (39.6±7.7 to 24.3±2.7 g/L, p=0.001), ferritin (65.7±54.8 to 30.3±20.4 ng/mL, p=0.01) and total protein (74.4±12.4 to 60.5±4.1 g/L, p=0.01) declined progressively. Folate dipped in the second trimester (14.8±8.2 ng/mL) before rising in the third. Serum iron and TS% increased significantly in the third trimester. Over 90% of participants had abnormal total protein or albumin levels by the third trimester.
Conclusions: Key nutritional biomarkers decline significantly in the second and third trimesters, supporting enhanced targeted nutritional monitoring and interventions in later pregnancy.

Keywords: Albumin anaemia, Folate, Iron, Micronutrients, Pregnancy, Protein, Vitamin B12

INTRODUCTION

Pregnancy induces profound physiological changes, including a significant expansion of plasma volume and increased demands for macro- and micronutrients to support fetal development. Among these, maintaining optimal iron status is paramount. Iron deficiency anemia (IDA) remains a major public health challenge, affecting a substantial proportion of pregnant women globally and contributing to adverse outcomes such as preterm birth, low birth weight, and increased maternal morbidity, particularly in low- and middle-income countries (LMICs).[1,2] A systematic review by Haider et al demonstrated that daily iron supplementation in

pregnancy, leading to improved hemoglobin levels, is associated with higher birth weights.[3]

Beyond iron, deficiencies in other essential micronutrients like folate, vitamin B12, and vitamin D are also prevalent and can result in a spectrum of adverse outcomes for both mother and child, including anemia, preeclampsia, and neural tube defects.[4,5] In sub-Saharan Africa, pregnant women face a heightened risk of these deficiencies due to a combination of factors, including poor dietary diversity and the increased metabolic demands of pregnancy.[6]

Ferritin, the primary iron-storage protein, is a key indicator of body iron stores.[7] However, iron metabolism during pregnancy is complex and influenced by dietary intake, absorption, and utilization.[8] While many studies have documented the general physiological declines in nutritional markers during pregnancy, few have comprehensively analyzed the trimester-specific trends and interrelationships between multiple parameters like ferritin, folate, vitamin B12, and protein status within the same cohort in the Nigerian context.[9-11] This study therefore aimed to: (1) assess trimester-specific changes in a panel of nutritional markers (iron studies, folate, vitamin B12, proteins) in pregnant women in Calabar, Nigeria, compared to non-pregnant controls; and (2) explore the correlations between these markers to understand their dynamic interplay during gestation.

METHODS

Study area and population

This longitudinal study was conducted at the Antenatal Care Clinic (ANC) of the University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State, Nigeria, between March, 2018 and March, 2019. UCTH is a major tertiary referral centre serving a diverse population. The study population consisted of pregnant women receiving ANC and a control group of non-pregnant women.

Study design and subject selection

A longitudinal design was employed to track nutritional parameters in pregnant women from the first to the third trimester. Participants were enrolled using a consecutive (non-probability) sampling method. This approach was chosen as it is practical for longitudinal studies with strict inclusion/exclusion criteria, allowing for the enrollment of all eligible women within the study period to observe intra-individual trends over time, rather than aiming for population-level generalizability.

Inclusion criteria

Inclusion criteria include (i) Confirmed pregnancy in the first trimester (≈10 weeks gestation), (ii) Age 18-41 years, (iii) Receiving ANC at UCTH, (iv) Willing to provide informed consent and be followed throughout pregnancy.

Exclusion criteria

Exclusion criteria include (i) Chronic medical conditions (hypertension, diabetes mellitus, chronic renal disease, sickle cell anemia, HIV infection), (ii) Multiple gestations, (iii) Therapeutic diets other than routine iron and folic acid supplementation.

The control group comprised 50 age- and parity-matched non-pregnant women, recruited from hospital staff and the community, who were not on any nutritional supplements and had no known chronic illnesses.

Sample size determination

The minimum sample size was calculated using the Cochran formula for longitudinal studies. Assuming a 20% attrition rate, a final sample of 80 pregnant women was targeted for enrollment in the first trimester.

Data and sample collection

At enrollment (10 weeks gestation, representing the 1st trimester), a structured questionnaire was administered to collect demographic data including age, education level, parity, household size, and monthly income. Anthropometric measurements (height and weight) were taken, and BMI was calculated. A 5 mL venous blood sample was collected from each participant. Two milliliters were dispensed into an EDTA tube for full blood count analysis, and 3 mL were dispensed into a plain tube. After 30 minutes of clotting, serum was separated by centrifugation, aliquoted, and stored at -20°C for subsequent biochemical analysis. This entire process was repeated for the pregnant participants during their 24-week (2^{nd} trimester) and 36-week (3^{rd} trimester) ANC visits.

Laboratory analysis

Hematologic parameters (hemoglobin, PCV, platelet count) were measured using a Sysmex KX-21N automated hematology analyzer. Serum levels of ferritin, folate, vitamin B12, and soluble transferrin receptors (sTfR) were determined using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Calbiotech, USA).

Competitive ELISA Principle (Folate, Vitamin B12, sTfR): These assays are based on a competitive binding principle. Biotinylated antibodies specific to the analyte of interest are mixed with the patient sample, allowing for antigen-antibody binding. This mixture is then added to streptavidin-coated microplate wells, immobilizing the antibody-bound fraction. An enzyme conjugate is added, which competes with the native antigen for binding sites. After incubation and washing, a substrate solution is added, producing a color whose intensity is *inversely* proportional to the concentration of the native antigen in the sample. A standard curve is generated to determine unknown concentrations.

Sandwich ELISA Principle (Ferritin): This assay uses a solid-phase sandwich method. Samples and a biotinylated anti-ferritin antibody are added to streptavidin-coated wells. After incubation and washing, an HRP-conjugated anti-ferritin antibody is added, forming a "sandwich" (antibody-ferritin-antibody). Following another wash, a substrate is added, and the color developed is directly proportional to the ferritin concentration in the sample.

Serum total protein and albumin were measured using standard colorimetric methods (Biuret and Bromocresol Green methods, respectively).[10] Serum iron and Unsaturated Iron-Binding Capacity (UIBC) were measured using colorimetric kits (Teco Diagnostics, USA). Total Iron-Binding Capacity (TIBC) was calculated as: TIBC (µg/dL) = Serum Iron (µg/dL) + UIBC (µg/dL). Transferrin Saturation (TS) was calculated as: TS (%) = (Serum Iron / TIBC) × 100.

Statistical analysis

Data were analyzed using SPSS version 26. Descriptive statistics (mean ± SD, frequencies, percentages) were used to summarize participant characteristics and nutritional parameters. Differences in means between pregnant women and non-pregnant controls were assessed using an independent samples t-test. Differences in means across the three trimesters were analyzed using repeated measures ANOVA, followed by post-hoc tests (Bonferroni) for pairwise comparisons. Pearson's correlation coefficient was used to explore relationships between nutritional markers. A p value <0.05 was considered statistically significant.

Ethical considerations

Ethical approval was obtained from the Health Research Ethical Committee of the University of Calabar Teaching Hospital (UCTH/HREC/33/372). All participants provided written informed consent before enrollment.

RESULTS

Demographic characteristics

A total of 72 pregnant women completed all three study visits and were included in the final analysis, alongside 50 age- and parity-matched non-pregnant controls. The baseline demographic characteristics of the pregnant participants are presented in Table 1.

The majority of participants (52.78%) were aged 18-29 years, with 47.22% in the 30-41 years age group. Most women had tertiary education (73.61%), while 26.39% had secondary education. Regarding parity, 41.67% were experiencing their first pregnancy, while 58.33% had two or more children. The majority (61.11%) lived in households with fewer than four members, while 38.89% had household sizes greater than four. In terms of monthly income, 43.05% were unemployed, 29.17% earned between ₦10,000-49,999, 9.72% earned between ₦50,000-99,999, and 18.05% earned above ₦100,000.

Table 1: Demographic characteristics of pregnant study participants.

Variables	Frequency (n=72)	Percentage (%)
Age group (years)		
18-29	38	52.78
30-41	34	47.22
Education		
Secondary	19	26.39
Tertiary	53	73.61
Parity		
Once	30	41.67
2 and above	42	58.33
Household size		
<4	44	61.11
>4	28	38.89
Average monthly income (Naira)		
10,000-49,999	21	29.17
50,000-99,999	7	9.72
>100,000	13	18.05
Unemployed	31	43.05

Table 2 presents the comparison of key nutritional indices between pregnant women across trimesters and the non-pregnant control group. Pregnant women in the second and third trimesters had significantly lower mean levels of albumin (28.8±10.4 g/l and 24.3±2.7 g/l, respectively) compared to non-pregnant women (43.5±2.7 g/l, p<0.05). Folate levels were significantly lower in the second trimester (14.8±8.2 ng/ml) compared to non-pregnant controls (20.1±9.5 ng/ml, p<0.05). Vitamin B12 levels were also significantly reduced in the second trimester (666.6±258.9 pg/ml) relative to non-pregnant women (871.9±279.5 pg/ml, p<0.05). Hemoglobin and packed cell volume (PCV) were significantly lower in all three trimesters of pregnancy compared to the non-pregnant state (p<0.05).

Within the pregnant cohort, significant changes were observed across trimesters for multiple nutritional parameters (Tables 3 and 4).

As shown in Table 3, total protein and albumin demonstrated consistent and significant declines from the first to the third trimester. Total protein decreased from 74.4±12.4 g/l in the first trimester to 60.5±4.1 g/l in the third trimester (p<0.01). Albumin declined progressively from 39.6±7.7 g/l in the first trimester to 24.3±2.7 g/l in the third trimester (p<0.01). Folate levels showed a significant dip in the second trimester (14.8±8.2 ng/ml) compared to the first trimester (18.9±10.4 ng/ml, p<0.05), before increasing again in the third trimester (20.1±7.8 ng/ml, p<0.01 vs. second trimester). Vitamin B12 levels

were lowest in the second trimester (666.6±258.9 pg/ml), though this did not reach statistical significance across all time points. Body mass index (BMI) increased steadily throughout pregnancy, peaking in the third trimester (30.5±5.2 kg/m², p<0.05 vs. first trimester).

Table 2: Comparison of key nutritional indices between pregnant and non-pregnant women.

Parameters	Non-pregnant (n=50)	1st Trimester (n=72)	2nd Trimester (n=69)	3rd Trimester (n=68)	F (p value)
Total Protein (g/l)	75.2±4.8	74.4±12.4	72.3±44.2	60.5±4.1*	5.34 (0.01)
Albumin (g/l)	43.5±2.7	39.6±7.7*	28.8±10.4*	24.3±2.7*	100.42 (0.01)
Folate (ng/ml)	20.1±9.5	18.9±10.4	14.8±8.2*	20.1±7.8#	5.11 (0.002)
Vitamin B12 (pg/ml)	871.9±279.5	788.1±317.4	666.6±258.9*	699.4±506.7	3.89 (0.01)
Hemoglobin (g/dl)	12.8±0.9	11.2±1.1*	10.8±1.0*	10.5±1.2*	42.16 (0.001)
PCV (%)	38.2±2.8	33.6±3.1*	32.1±2.9*	31.5±2.4*	38.74 (0.001)

*Data are presented as Mean±SD. *Significantly different from non-pregnant group (p<0.05); #Significantly different from 2nd Trimester (p<0.01). PCV = Packed Cell Volume

Table 3: Trimester-specific trends in protein, vitamins, and BMI.

Parameters	1st Trimester (n=72)	2nd Trimester (n=69)	3rd Trimester (n=68)	P value (ANOVA)
Total protein (g/l)	74.4±12.4	72.3±44.2	60.5±4.1**	0.01
Albumin (g/l)	39.6±7.7	28.8±10.4*	24.3±2.7**	0.01
Folate (ng/ml)	18.9±10.4	14.8±8.2*	20.1±7.8##	0.002
Vitamin B12 (pg/ml)	788.1±317.4	666.6±258.9	699.4±506.7	0.01
BMI (kg/m²)	28.4±4.9	29.5±5.1	30.5±5.2*	0.01

*Data are presented as Mean±SD. *p<0.05 vs. 1st Trimester; **p<0.01 vs. 1st Trimester; ##p<0.01 vs. 2nd Trimester. BMI = Body Mass Index

Table 4 presents the trimester-specific trends for iron parameters. Serum ferritin decreased progressively across gestation, with significant reductions in both the second (34.2±41.9 ng/ml, p<0.01) and third (30.3±20.4 ng/ml, p<0.01) trimesters compared to the first trimester (65.7±54.8 ng/ml). Serum iron and transferrin saturation (TS%) followed a similar pattern: both decreased in the second trimester (70.3±30.0 µg/dl and 13.6±5.6%, respectively) before increasing significantly in the third trimester (121.5±20.7 µg/dl and 24.4±3.7%, respectively; p<0.01 for both compared to first and second trimesters). Conversely, unsaturated iron binding capacity (UIBC) increased in the second trimester (446.2±44.1 µg/dl, p<0.01 vs. first trimester) and fell significantly in the third trimester (377.8±47.6 µg/dl, p<0.01 vs. second trimester). Total iron binding capacity (TIBC) and soluble transferrin receptor (sTfR) remained relatively stable across all three trimesters, with no statistically significant variations.

Table 4: Trimester-specific trends in iron parameters.

Parameters	1st Trimester (n=72)	2nd Trimester (n=69)	3rd Trimester (n=68)	P value (ANOVA)
Ferritin (ng/ml)	65.7±54.8	34.2±41.9**	30.3±20.4**	0.01
Serum Iron (µg/dl)	89.9±23.0	70.3±30.0**	121.5±20.7**##	0.01
UIBC (µg/dl)	418.9±38.4	446.2±44.1**	377.8±47.6**##	0.01
TIBC (µg/dl)	507.9±39.3	515.4±42.5	499.3±53.8	0.19
TS (%)	17.4±4.3	13.6±5.6**	24.4±3.7**##	0.01
sTfR (nmol/ml)	23.9±7.6	22.9±5.5	23.6±6.9	0.42

*Data are presented as Mean±SD. **p<0.01 vs. 1st Trimester; ##p<0.01 vs. 2nd Trimester. UIBC = Unsaturated Iron Binding Capacity; TIBC = Total Iron Binding Capacity; TS = Transferrin Saturation; sTfR = Soluble Transferrin Receptor

Age-related differences in nutritional parameters were minimal. In the second trimester, women aged 30-34 years had significantly higher serum iron levels (74.84±33.15 µg/dl) compared to those aged 18-29 years (66.61±27.01 µg/dl, p=0.003), while younger women had higher sTfR levels (456.37±38.22 nmol/ml vs. 433.77±48.15 nmol/ml, p=0.002). In the third trimester, younger women (18-29 years) demonstrated significantly higher vitamin B12 levels than older women (30-34 years) (p=0.015).

Regarding parity, multiparous women (parity ≥2) had significantly higher BMI (29.07±5.69 kg/m² vs. 27.33±3.45 kg/m², p=0.047) and folate levels (p=0.037) in the first trimester compared to primigravidae. In the third trimester, transferrin saturation was significantly higher among multiparous women (31.39±6.00%) compared to women experiencing their first pregnancy (29.47±3.63%, p=0.045).

Table 5 shows the proportion of pregnant women with abnormal values for key nutritional markers across trimesters, based on standard clinical cutoffs. The prevalence of anaemia (Hb <11 g/dl) decreased slightly from 36.1% in the first trimester to 26.5% in the third trimester. However, the proportion of women with abnormally low total protein (<66 g/l) increased dramatically from 15.3% in the first trimester to 91.2% by the third trimester. Similarly, hypoalbuminaemia (albumin <35 g/l) affected 98.5% of women by the third trimester, compared to only 19.4% in the first trimester. Overt folate deficiency (<3.2 ng/ml) was rare throughout pregnancy (0-1.5%). Vitamin B12 deficiency (<200 pg/ml) increased from 2.8% in the first trimester to 7.4% in the third trimester. Low ferritin levels (<15 ng/ml) were observed in 5.6%, 14.5%, and 11.8% of women in the first, second, and third trimesters, respectively.

Table 5: Proportion of participants with abnormal nutritional status across trimesters.

Variable (cutoff for abnormality)	1st Trimester (n=72), N (%)	2nd Trimester (n=69), N (%)	3rd Trimester (n=68), N (%)
Anaemia (Hb <11 g/dl)	26 (36.1)	28 (40.6)	18 (26.5)
Total Protein (<66 g/l)	11 (15.3)	25 (36.2)	62 (91.2)
Albumin (<35 g/l)	14 (19.4)	51 (73.9)	67 (98.5)
Folate (<3.2 ng/ml)	1 (1.4)	1 (1.5)	0 (0.0)
Vitamin B12 (<200 pg/ml)	2 (2.8)	2 (2.9)	5 (7.4)
Ferritin (<15 ng/ml)	4 (5.6)	10 (14.5)	8 (11.8)

Hb = Hemoglobin, Cutoff values based on WHO guidelines for pregnancy

Table 6: Correlations between key nutritional parameters across trimesters and in non-pregnant women.

Parameter pair	1st Trimester (n=72)	2nd Trimester (n=69)	3rd Trimester (n=68)	Non-pregnant (n=50)
	r (p value)	**r (p value)**	**r (p value)**	**r (p value)**
Ferritin vs. Albumin	0.184 (0.121)	0.460 (0.000)**	0.162 (0.188)	0.235 (0.100)
Ferritin vs. BMI	0.251 (0.034)*	-0.099 (0.418)	0.033 (0.791)	0.002 (0.989)
Ferritin vs. Serum Iron	-0.143 (0.230)	-0.300 (0.012)*	-0.092 (0.455)	-0.148 (0.305)
Ferritin vs. TS%	-0.166 (0.164)	-0.306 (0.011) *	0.020 (0.870)	-0.135 (0.351)
Serum Iron vs. TIBC	0.355 (0.002)**	0.315 (0.008)**	0.479 (0.000)**	0.565 (0.000)**
Serum Iron vs. TS%	0.940 (0.000)**	0.977 (0.000)**	0.780 (0.000)**	0.906 (0.000)**
Ferritin vs. Vitamin B12	0.228 (0.054)	-0.078 (0.527)	0.130 (0.291)	0.369 (0.008)**

**Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level. BMI = Body Mass Index; TS% = Transferrin Saturation; TIBC = Total Iron Binding Capacity

Table 6 presents the correlations between key nutritional parameters across trimesters and in non-pregnant women. In the second trimester, ferritin demonstrated significant negative correlations with serum iron (r = -0.300, p=0.012) and transferrin saturation (r = -0.306, p=0.011), suggesting mobilization of iron stores during this period. A significant positive correlation was observed between ferritin and albumin in the second trimester (r = 0.460, p=0.000), and between ferritin and BMI in the first trimester (r = 0.251, p=0.034). In non-pregnant women, ferritin correlated positively with vitamin B12 (r = 0.369, p=0.008).

As expected, serum iron showed strong positive correlations with transferrin saturation across all trimesters and in non-pregnant women (r ranging from 0.780 to 0.977, all p<0.01). Serum iron also correlated positively with TIBC in all groups (r ranging from 0.315 to 0.565, all p<0.01).

DISCUSSION

This longitudinal cohort describes trimester-specific behaviour of protein and iron-related indices among pregnant women in Calabar, adding context-specific data from South-South Nigeria. The demographic profile of our cohort (Table 1) reveals a predominantly educated (73.61% tertiary), multiparous (58.33%) population with a significant proportion (43.05%) reporting unemployment, which may influence dietary access and nutritional status during pregnancy.

We observed progressive reductions in albumin and ferritin with nadirs in late gestation, patterns consistent with physiological hemodilution, increasing iron utilization for red cell mass expansion, and fetal demand.[12-16] As shown in Tables 3 and 4, albumin declined from 39.6±7.7 g/l in the first trimester to 24.3±2.7 g/l in the third trimester (p<0.01), while ferritin decreased from 65.7±54.8 ng/mL to 30.3±20.4 ng/mL (p<0.01). The rise in UIBC during the second trimester (446.2±44.1 µg/dL) followed by a fall in the third (377.8±47.6 µg/dL), together with increases in serum iron and transferrin saturation (TS%) by late pregnancy (121.5±20.7 µg/dL and 24.4±3.7%, respectively), is biologically plausible as iron transport and utilization adapt to gestational needs.[4,6,14,17] Overall, these trimester trends align with the established

physiology of normal pregnancy while providing local data to inform antenatal monitoring in this setting.[12,15,18]

Our data also show second trimester dips in folate and vitamin B12 (Table 3), which fit with peak requirements for DNA synthesis and erythropoiesis in mid gestation.[19-21] Folate levels decreased from 18.9±10.4 ng/mL in the first trimester to 14.8±8.2 ng/mL in the second trimester ($p<0.05$), while vitamin B12 reached its nadir of 666.6±258.9 pg/mL during the same period. Although overt folate/B12 deficiency was uncommon in this cohort (Table 5), affecting less than 3% of participants across all trimesters, likely reflecting routine antenatal supplementation and diet, the lower mid-gestation levels support continued emphasis on compliance counselling during ANC visits.[8,22-24] This is particularly relevant given the socioeconomic diversity of our cohort, where 43% are unemployed and may face financial barriers to optimal nutrition. From a service delivery standpoint, maintaining guideline iron-folate intake and reinforcing dietary diversity remain appropriate, rather than proposing new therapeutic strategies based solely on these biochemical trends.[10,25]

Contrary to some reports, TIBC did not differ significantly across trimesters in our study (Table 4), which may reflect differences in study design, measurement timing, diet/infection burden, or sample size/power relative to previous studies that found trimester-related shifts in transport capacity.[14,26] Given the well-known acute phase reactivity of ferritin, interpretation of depleted iron stores is more robust when inflammation markers (e.g., CRP or AGP) are available; these were not collected here and should be incorporated in future work to refine iron status classification.[12,16,27] However, our concurrent measurement of sTfR which is less affected by inflammation partially mitigates this limitation, as sTfR remained stable across trimesters (Table 4), suggesting the ferritin decline reflects true store depletion rather than inflammatory redistribution.[7,13,28] Future work should incorporate both inflammatory markers and sTfR to strengthen iron status assessment in this population.

The correlation patterns in our cohort are internally consistent with iron physiology (Table 6). In the second trimester, ferritin correlated negatively with serum iron ($r=-0.300$, $p=0.012$) and TS% ($r=-0.306$, $p=0.011$), suggesting dynamic mobilization of stores and changes in transport saturation as gestation progresses. Positive correlations between ferritin and albumin ($r=0.460$, $p=0.000$) in the second trimester and ferritin and BMI ($r=0.251$, $p=0.034$) in the first trimester point to broader links between nutritional status and iron stores in this setting.[11, 29] These relationships, while moderate, reinforce the clinical value of a panel-based rather than single marker approach to maternal iron assessment.[17,18,30]

Age and parity-related differences, though minimal, warrant mention. Younger women (18-29 years) in the third trimester had significantly higher vitamin B12 levels than older women (30-41 years, $p=0.015$), which may reflect cumulative nutritional depletion in older or multiparous women.[19,31] Multiparous women demonstrated higher BMI and folate levels in the first trimester ($p<0.05$), possibly indicating greater nutritional awareness from previous pregnancies, while their higher transferrin saturation in the third trimester ($p=0.045$) suggests enhanced iron mobilization. These observations, though derived from subgroup analyses, support consideration of parity and age when interpreting nutritional status in pregnancy.

Within routine ANC, our findings support three practical actions: (i) continued monitoring of haemoglobin and key indices (albumin, ferritin, iron/TIBC/UIBC, TS%), (ii) reinforcement of iron–folate supplementation and nutrition counselling, especially through mid-pregnancy, and (iii) consideration of contextual factors (diet, infection exposure, parity, socioeconomic status) when interpreting laboratory values.[26,32] The dramatic increase in the proportion of women with abnormal protein status by the third trimester (Table 5) affecting over 90% for total protein and 98% for albumin highlights a potential functional protein deficiency that may contribute to complications such as edema and deserves clinical attention.[16,27,33] This finding is particularly significant given that 43% of our cohort reported unemployment, which may limit access to protein-rich foods. Linking these biochemical measures to maternal and neonatal outcomes (e.g., preterm birth, low birth weight, peripartum transfusion) will be essential to strengthen clinical meaning and determine thresholds that best predict risk in this population.[7,15,34]

Trimester-associated declines in ferritin and haemoglobin have been consistently described across regions and populations, attributed to hemodilution and increased fetal demand.[15,29] Reports on TIBC/TS% are more heterogeneous, varying with supplementation, inflammation, and analytic timing.[14,31] Declines in albumin with advancing gestation are likewise well documented, reflecting plasma volume expansion; very low albumin may influence oncotic pressure and fluid balance, although most healthy pregnancies tolerate these changes without adverse effects.[25,30] Our results fall within these expected patterns while providing data specific to Calabar that can inform local ANC practice.

This study has few strengths. The study provides longitudinal, trimester-specific data from South-South Nigeria and concurrently profiles ferritin, sTfR, iron transport indices, albumin/total protein, folate, vitamin B12, and BMI, with a non-pregnant comparator group for contextual interpretation. The inclusion of comprehensive demographic data (Table 1), including education, parity, household size, and income, enhances the interpretability of findings and allows for consideration of socioeconomic factors in nutritional assessment.

This study has few limitations. Single centre design with consecutive sampling (potential selection bias); smaller than planned sample size (≥80 target vs 72 at baseline); absence of inflammation markers to qualify ferritin; no adjustment for multiple testing despite numerous comparisons; and lack of maternal or neonatal outcomes, which limits clinical inference and generalizability. These are important considerations when interpreting the findings and should be addressed in future research.

CONCLUSION

In this longitudinal cohort from Calabar, we observed trimester-related trends in key nutritional indices: ferritin and albumin declined progressively across gestation with lowest values in the third trimester, while folate and vitamin B12 were lowest in mid-pregnancy; serum iron and transferrin saturation increased by late gestation. Haematologic parameters remained lower than in non-pregnant controls but showed limited trimester variation. The dramatic rise in abnormal protein status by the third trimester (affecting >90% of participants) highlights a previously underappreciated nutritional vulnerability in this population, potentially linked to the high proportion (43%) of unemployed women with limited financial access to protein-rich foods. Collectively, these patterns are consistent with physiological hemodilution and increased nutrient demand of pregnancy, though the magnitude of protein decline suggests a need for clinical attention. The findings support routine antenatal monitoring of haemoglobin and selected biochemical markers and reinforcement of guideline iron–folate supplementation and nutrition counselling within ANC services, with particular attention to women of lower socioeconomic status. Future studies in this setting should include inflammation markers for ferritin interpretation, richer dietary data, multiplicity considerations, probability-based or multi-centre sampling, and linkage to maternal–neonatal outcomes to strengthen clinical applicability.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Abel R, Rajaratnam J, Gnanaskarana VJ, Jayaram P. Prevalence of anemia and iron deficiency in three trimesters in Rural Vallare District, South India. Trop Doct. 2001;31(12):86-9.
2. Okafor IM, Oko JO, Njar VE, Emechebe C. Serum iron, total iron binding capacity and malaria parasite density of pregnant women with asymptomatic malaria parasite infection attending antenatal clinic at a tertiary hospital in Calabar, Nigeria. Afr J Feto-Matern Med. 2024;3(2):84-90.
3. Alper BS, Kimber R, Reddy AK. Using ferritin levels to determine iron-deficiency anemia in pregnancy. J Fam Pract. 2000;49(9):829-32.
4. Amah-Tariah FS, Ojeka SO, Dapper DV. Haematological values in pregnant women in Port Harcourt, Nigeria II: Serum iron and transferrin, total iron and unsaturated iron binding capacity, and some red cell and platelet indices. Niger J Physiol Sci. 2011;26:173-8.
5. Chaudhari SJ, Bodat RK. Tracking of haematological parameters in first and second trimesters. Natl J Med Res. 2015;5(3):259-61.
6. Dapper DV, Ibe CJ, Nwauche CA. Haematological values in pregnant women in Port Harcourt, Nigeria. Niger J Med. 2006;15(3):237-40.
7. Okafor IM, Okpokam DC, Antai AB, Usanga EA. Soluble transferrin receptor as a marker in the diagnosis of iron deficiency anaemia: a study in Calabar. Int J Biomed Lab Sci. 2014;3:40-6.
8. Hytten F. Blood volume changes in normal pregnancy. In: Letsky EA, editor. Hematological Disorders in Pregnancy. London: WB Saunders; 1985:601-612.
9. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. BMJ. 2013;347.
10. Kiely ME, McCarthy EK, Hennessy Á. Iron, iodine, and vitamin D deficiencies during pregnancy: epidemiology, risk factors, and developmental impacts. Proc Nutr Soc. 2021;80(3):290-302.
11. Favier A, Ruffieux D. Physiological variations of serum levels of copper, zinc, iron, and manganese. Biomed Pharmacother. 1983;37(9-10):462-6.
12. Gibson RS, Abebe Y, Stabler S, Allen RH, Westcott JE, Stoecker BJ, et al. Zinc, gravida, infection, and iron but not vitamin B-12 or folate status predict hemoglobin during pregnancy in Southern Ethiopia. J Nutr. 2008;138(3):581-6.
13. Horton DK, Adetona O, Aguilar-Villalobos M, Cassidy BE, Pfeiffer CM, Schleicher RL, et al. Changes in biochemical indicators and diet and nutritional status of pregnant women across trimesters in Trujillo, Peru, 2004–2005. J Nutr. 2013;12(1):80.
14. Okafor IM, Ogar CO, Okoroiwu HU. Serum ferritin and asymptomatic malaria: a study in Calabar, Cross River State. Afr J Lab Haematol Blood Transfus Sci. 2023;2(1):15-22.
15. Lee HS, Kim MS, Kim MH, Kim YJ, Kim WY. Iron status and its association with pregnancy outcome in Korean pregnant women. Eur J Clin Nutr. 2006;60(9):1130-5.
16. Letsky EA. The hematological system. In: Broughton PF, Chamberlain GVP, editors. Clinical Physiology in Obstetrics. 3rd ed. Oxford: Blackwell; 1998:71-110.
17. Okafor IM, Antai AB, Usanga EA. Evaluation of sTfR, sTfR:SF and other iron-related parameters of pregnant women in Cross River State. Trop J Med Res. 2017;20(2):180-4.
18. Okafor IM, Mbah M, Usanga EA. The impact of anaemia and malaria parasite infection in pregnant women: Nigerian perspective. J Dent Med Sci. 2012;1(1):34-8.

19. Singh AS, Masuku MB. Sampling techniques and determination of sample size in applied statistics research: an overview. Int J Econ Commer Manag. 2014;2(11):1-22.
20. Raza N, Sarwar I, Munazaa B, Ayub M, Suleman M. Assessment of iron deficiency in pregnant women by determining iron status. J Ayub Med Coll Abbottabad. 2011;23(2):36-40.
21. Okafor IM, Asemota EA, Antai AB, Usanga EA. Prevalence of iron deficiency anaemia among pregnant women in Calabar, Cross River State, Nigeria. J Pharm Biol Sci. 2013;7(2):60-4.
22. Tietz NW, Rinker AD, Morrison SR. When is a serum iron really a serum iron? The status of serum iron measurements. Clin Chem. 1994;40(4):546-51.
23. Koenig MD, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. Nutrients. 2014;6(8):3062-83.
24. Leonard AJ, Chalmers KA, Collins CE, Patterson AJ. The effect of nutrition knowledge and dietary iron intake on iron status in young women. Appetite. 2014;81:225-31.
25. Okafor IM, Akpan PA, Usanga EA. Prevalence and types of anaemia in malaria-infected pregnant women attending antenatal clinic in University of Calabar Teaching Hospital, Calabar, Nigeria. J Nat Sci Res. 2012;2(7):73-8.
26. Okafor IM, Ibanga IA, Asuquo IJ. Prevalence of anaemia and iron deficiency anaemia in a Nigerian rural community. Sokoto J Med Lab Sci. 2018;3(2).
27. Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe J. Williams Obstetrics. 24th ed. New York: McGraw-Hill Education; 2014.
28. Rasmussen KM, Yaktine AL. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press; 2016.
29. Allen LH. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr. 2008;138(12):2523-2.
30. Karaoglu L, Pehlivan E, Egri M, Deprem C, Gunes G, Genc MF, Temel I. The prevalence of nutritional anemia in pregnancy in an east Anatolian province, Turkey. BMC Public Health. 2010;10:329.
31. Molloy AM, Kirke PN, Scott JM. Folate, vitamin B12, and neural tube defects. Int J Epidemiol. 2017;47(1):67-74.
32. Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr. 2005;81(5 Suppl):1218S-22S.
33. Food and Agriculture Organization. Minimum dietary diversity for women: a guide for measurement, 2016. Available at: https://openknowledge.fao.org/server/api/core/bitstreams/d5943472-fbfb-4e58-9137-eb0628ec4392/content. Accessed 01 January 2026.
34. World Health Organization. Guideline: daily iron and folic acid supplementation in pregnant women, 2020. Available at: https://www.who.int/tools/elena/interventions/daily-iron-pregnancy. Accessed 01 January 2026.

Cite this article as: Igwilo AC, Okafor IM, Amilo GI. Trimester specific changes in nutritional and iron related biomarkers among pregnant women in Calabar, Nigeria: a longitudinal study. Int J Res Med Sci 2026;14:3745-52.