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Maternal Hypovitaminosis D and Iron Homeostasis Biomarkers: Correlation and Regression Analysis in Iraqi Pregnant Women

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ABSTRACT:

Background: Vitamin D and iron deficiencies frequently coexist during pregnancy, yet their clinical crosstalk remains debated. This study aimed to evaluate the diagnostic and predictive associations between maternal serum Vitamin D levels and key iron homeostasis biomarkers.

Subjects and Methods: A cross-sectional study was conducted on 196 pregnant women (N = 196; mean age 26.8 ±6.1years). Serum Vitamin D, ferritin, total iron-binding capacity (TIBC), erythropoietin (EPO), and transferrin levels were quantified alongside socio-demographic and obstetric parameters. Data were analyzed using Spearman correlation, multiple linear regression, and receiver operating characteristic (ROC) curves.

Results: Overall, 63.8% (n = 125) of participants exhibited Vitamin D inadequacy (<30ng/mL). Vitamin D3 showed no significant linear correlations with ferritin (r = 0.064), transferrin (r = 0.053), or EPO (r = 0.031), though a trend with TIBC emerged (r = -0.150, p = 0.069). Ferritin inversely correlated with EPO (r = -0.270, p = 0.010). After controlling for confounders, Vitamin D was not an independent predictor of any iron biomarker (all p > 0.05). ROC analyses confirmed poor diagnostic performance for all iron markers in predicting Vitamin D status (AUC = 0.413–0.587, all p > 0.05).

Conclusion: Serum iron homeostasis biomarkers do not reliably reflect or predict maternal Vitamin D status, highlighting the need for independent screening of both micronutrients during antenatal care.

1. INTRODUCTION

Vitamin D is a steroid hormone that helps to maintain calcium homeostasis and facilitates bone mineralization in the body. Vitamin D deficiency during pregnancy can cause bone loss, preeclampsia, preterm delivery, and low birth weight (Mulligan et al., 2010). Iron-deficiency anemia is another common nutritional-deficiency disease during pregnancy. Under normal circumstances, serum ferritin concentrations can be indicative of the total amount of iron stored in the body. While there are many iron-rich foods such as red meat, livers, nuts, and green, leafy vegetables, the food sources of vitamin D are relatively limited (Benedik, 2022). In humans, vitamin D is primarily produced from the skin by way of exposure to ultraviolet light. However, due to the increased nutritional needs for body metabolism and fetal growth, pregnant women are advised to take daily vitamin D supplements (Holick et al., 2011). A previous study showed that the most effective and safest dose for achieving optimal serum vitamin D levels among pregnant women in all races is 4000 IU per day (Hollis et al., 2011). It is

however not clear if vitamin D supplementation is still required for healthy women who have adequate dietary intake to obtain sufficient vitamin D during pregnancy.

Previous research has shown no meaningful differences in iron status improvement among women receiving different doses of vitamin D supplementation during gestation (4200 IU, 16,800 IU, and 28,000 IU per week) (O'Callaghan et al., 2020). While some evidence shows that vitamin D supplementation cannot improve serum ferritin concentrations in pregnant women (O'Callaghan et al., 2020, Braithwaite et al., 2019, Smith et al., 2017), low vitamin D status was found to be associated with an increased risk of prenatal iron deficiency (Thomas et al., 2015). Notably, although previous research has examined the effect of vitamin D supplementation on both vitamin D and ferritin concentrations, it remains unclear whether vitamin D supplementation could have simultaneous effects on both vitamin D and iron levels. Furthermore, while natural food are important sources of iron, little work has been undertaken to examine the effect of diet and its potential interaction with vitamin D supplementation on vitamin D status, particularly during pregnancy (Wong et al., 2022).

There are two major types of vitamin D, a steroid: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (Omar et al., 2025). Skin synthesizes approximately 80% of vitamin D3 via UVB radiation (295–315 nm), with the rest 20% obtained from food (Pilz et al., 2018). Fatty fish, cod liver oil, and egg yolks are some examples of animal sources of vitamin D3, whereas plants, fungus, and yeast produce vitamin D2 upon exposure to UV light (Roseland et al., 2018). The liver converts all forms to 25(OH)D by means of 25-hydroxylase enzymes, whereas in the kidneys, 1-alpha-hydroxylase transforms them into their active form, 1,25-dihydroxyVitamin D3 (CYP27B1) (Roseland et al., 2018, Pilz et al., 2018). Study in Malawi found that among 150 pregnant women, 32% had iron deficiency and deficiency of one or more micronutrients such as vitamin D (van den Broek and Letsky, 2000). Vitamin D3 deficiency is a global public health issue that has been linked to several adverse health consequences, including pregnancy outcomes (Kaludjerovic and Vieth, 2010).

The high level of serum transferrin is the most important factor related to anemia in the third trimester, followed by the low level of hepcidin. Low cholecalciferol level tends to favor the incident of anemia (Judistiani et al., 2020). In a population with concurrently high prevalence rates of iron and vitamin D deficiency, prenatal vitamin D supplementation did not lead to improvements in iron status by late gestation (O'Callaghan et al., 2020).

In pregnant women, transferrin saturation, as a measure of the amount of iron delivered to tissues, was positively associated with a modulation in the status of vitamin D3 above >20 ng/mL, which indicated improved iron supply to tissues in women (Blanco-Rojo et al., 2013).

The WHO set a Global Nutrition Target of a 50% reduction in anemia in women of reproductive age, by 2025. Later this target was extended to 2030 (WHO, 2014). In fact, millions of women throughout the world, most of them in low- and middle-income countries, are still afflicted with anemia and vitamin D3 deficiency, which reduces economic productivity and increases all-cause mortality and morbidity, besides having adverse effects on expecting mothers and newborns (WHO, 2014).

Although currently, there is no clinical evidence to guide, the evidence does indicate a biologically plausible relationship between vitamin D3 status and iron homeostasis in pregnancy. Well-designed studies are thus needed that detail baseline deficiency, employ appropriate Vitamin D3 dosing, and measure a panel of anemia-related biochemical parameters, including hepcidin, to determine whether or not vitamin D3 should be implemented as part of antenatal strategies to prevent or treat anemia. The present investigation represents the first to examine the interrelations among anemia, iron status, and VD Deficiency in fertile women in Iraq. This investigation examined whether vitamin D deficiency represents a modifiable risk factor contributing to iron deficiency and anemia rates using an integrated assessment approach by taking into consideration the known determinants. The study aimed to investigate correlation of serum vitamin D3 in pregnant women with some iron-related biomarkers, including serum ferritin, serum iron, total iron-binding capacity (TIBC), Erythropoietin (EPO), serum transferrin. .

Study hypothesized (Null Hypothesis) that serum Vitamin D will not be statistically correlated with iron related biochemical markers during pregnancy.

2. MATERIALS AND METHODS

2.1. Study Design, Setting, Period, and Sampling

This is an Observational, Analytical, Cross-Sectional Study entitled **"Maternal Hypovitaminosis D and Iron Homeostasis Biomarkers: Correlation and Regression Analysis in Iraqi Pregnant Women".** The data and blood collection was conducted from June 1st, 2024, to January 1st; 2025.four health care centers were selected from four geographical zones (central, northern, southern, and western) using a convenience sampling technique to ensure proportional representation. (vitamin D3 adequacy≥30ng/dL and vitamin D3 Inadequacy<30ng/dL)

2.2. Sample Size

A total of 211 pregnant women were initially approached through convenience sampling from the four selected centers. From these, 196 pregnant met the inclusion criteria and identified as our study sample. This final sample size (N = 196) provided adequate statistical power. It was conducted as a part of the vitamin D status in pregnancy.

2.3. Sample Size Determination

Determination the minimum required sample size for this study was determined a priori utilizing (G*Power) software (Version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany) configured for multiple linear regression models (Fixed effects, R2 deviation from zero). The statistical parameters were established based on standard epidemiological research metrics and biological assumptions: effect Size (f2): 0.15 was selected, reflecting the expected physiological contribution of a single continuous micronutrient (Vitamin D3) to dynamic systemic hematological parameters when controlled alongside complex maternal physiological adaptions. Alpha (α) = 0.05. Statistical Power (1 - β) = 0.90.

2.4. Inclusion/exclusion

These criteria included pregnant women who were residents of the population or resided at Koya district or sub-urban for more than 6 months who were registered at the Health Service Centers. Study included pregnant women without consider trimesters. All were excluded non pregnant women and those had previously chronic disease as diabetes, hypertension, and if blood sample hemolysis. Pregnant women were particularly selected because of the high percentage of vitamin D among pregnant women.

2.5. Study sample and ethical approval

The ethical committee at Koya University approved this study, after getting ethical clearance from the Ethics Committee of the Faculty of Science and Health, Koya University, through Ethics Form 010 Bio on the 11th of March 2024.

2.6. Study tools and data collection, procedure, and method

The questionnaire was divided into two main sections. The first section collected socio-demographic information about the pregnant, including socio-demographic variables like age, education level, and parital status were taken through a structured questionnaire. Height and weight of the subjects were taken using a device of a different brand but the same model for calculating the BMI of the subjects. The second part was included datas from laboratory after performing chemical tests for inflammatory parameters. After an overnight fasting state, the venous blood (5 mL) of the women was then collected. This was poured into gel tubes for the separation of serum after the centrifuge at 1800x for 10 min. Serum was aliquoted in five Eppendorf tubes and stored in the deep freezer at -20°C until the biochemistry test analysis. Immunochemolumescence assay analyzer (Maglumi X8) was used for biochemical testing. Measure the levels of vitamin D3, EPO, and ferritin in the blood. Serum TIBC was then tested using (Mindray chemical analyzer, China). Elisa was used for determination of transferrin.

2.7. Statistical Analysis

Data cleaning, management, and statistical modeling were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). The "Normality of Residuals" Anderson-Darling, Shapiro-Wilk and Kolmogorov-Smirnov test result output showed that the data was not passed normally distributed ($p<0.05$). Spearman rank correlation coefficient r ranging from -1 to 1. Coefficient was used to test correlation of non-parametric variables. The multiple linear regression analysis tests used to estimate the associations between serum vitamin D3 concentrations with thyroid function markers independently. Continuous variables were summarized using means, standard deviations (Jarmund et al.), standard errors (SE), skewness, and kurtosis. Categorical variables were presented as absolute frequencies (N) and percentages (%). Multiple linear regression analyses were performed to evaluate the independent predictive capacity of serum Vitamin D3 on iron profile parameters. Each model was adjusted for potential socio-demographic and clinical confounders: maternal age,

pregnancy period, BMI, education level, occupation, physical activity, place of residence, economic status, gestational trimester, and parity status. Multicollinearity was assessed across all regression models using the Variance Inflation Factor (VIF). All VIF values remained well below the conservative threshold of 5.0 (range: 1.03 to 4.75), indicating no distorting multicollinearity. Receiver Operating Characteristic (ROC) curve analyses were executed to evaluate the capacity of serum iron profiles to discriminate across distinct clinical categories (adequacy, and Inadequacy). standard errors, and two-tailed p-values were computed. All statistical tests were two-tailed, and statistical significance was set at alpha = 0.05 ($p < 0.05$).

3. RESULTS

3.1. Socio-demographic and Obstetric Profiles of the Study Cohort

The study included a final cohort of 196 pregnant women (N = 196). Socio-demographic and clinical distributions across the sample are detailed in Table 1. The mean age of participants was 26.8 ± 6.1 years (range: 15–42 years). Overall, 63.8% (n = 125) of participants exhibited serum Vitamin D3 Inadequacy (<30 ng/mL), whereas 36.2% (n = 71) demonstrated Vitamin D sufficiency (30 ng/mL).

Table1: *Socio-Demographic and Baseline Clinical Characteristics of the Study Cohort (N = 196)*

Variables	Categories	Frequency (n)	Percentage (%)
Vitamin D3 status	Inadequacy ≤30	125	63.8
	Adequacy >30	71	36.2
Trimester status	First Trimester	25	12.8
	Second Trimester	116	59.2
	Third trimester	55	28.1
Education level	Illiterate	22	11.2
	primary	31	15.9
	secondary	39	19.9
	High school	44	22.4
	Above high school	60	30.6
BMI status	Normal weight	49	24.5
	Over weight	104	53.1
	Obese	43	21.9
Parital state	Primigravida	101	51.5
	multigravida	95	48.5
Age groups	Under 21 years	24	12.24
	21 to 30 years	131	66.83
	31 to 40 years	36	18.36
	More than 40 years	5	2.55
State of residence	Urban	156	79.6
	Rural/Semi-Urban	40	20.4

3.2. Descriptive Statistics and Distributional Profiles of Parameters

Table 2 presents the mean, standard deviation (S.D.), standard error, skewness, and kurtosis values for maternal plasma Vitamin D3, hematological markers, and iron homeostasis regulators across the study cohort (N = 196). The mean maternal Vitamin D3 concentration was 23.534 ± 14.271 ng/mL, structurally corroborating the high prevalence of insufficiency noted in the baseline cohort characteristics. Evaluation of iron reserve architecture revealed a mean ferum Ferritin level of 27.352 ±30.188 mg/L, which sits below the clinical cutoff of 30 mg/L for absolute iron deficiency during gestation. Distributional analysis via skewness and kurtosis coefficients indicated varying degrees of non-normality across parameters. Serum Ferritin (skewness = 1.874), EPO (skewness = 2.347), and Serum Transferrin (skewness = 3.624, kurtosis = 15.943) exhibited severe rightward skewness, highlighting notable individual variations and outlier concentrations within these iron mobilization networks.

Table2: Descriptive Statistics (N = 196)

Variable	Mean ± S.D.	Standard error	Skewness	Kurtosis
Vitamin D3 ng/mL	23.534±14.271	1.960	0.869	0.733
Ferritin µg/L	27.352±30.188	4.146	1.874	3.138
TIBC µg/dL	312.257±23.628	3.245	-0.912	2.584
EPO mIU/mL	53.462±37.336	5.128	2.347	8.123
transferrin µg/mL	1.158±1.605	0.220	3.624	15.943

3.3. Correlation of vitamin D3 with iron and iron related markers

However, Vitamin D3 showed no significant linear relationship with cellular storage pools like Serum Ferritin ($r = 0.064$, $p = 0.372$) or core transport vehicles like Serum Transferrin ($r = 0.053$, $p = 0.551$). It did demonstrate a marginal inverse trend with Total Iron-Binding Capacity (TIBC) ($r = -0.150$, $p = 0.069$) and cellular though neither breached the standard significance threshold ($p < 0.05$). Serum Ferritin stores were directly tied to circulating EPO ($r = -0.270$, $p = 0.010$).

Table3: Spearman Rank Correlation Matrix of Maternal Serum Vitamin D3 Levels and Systemic Iron Status Biomarkers (N = 196)

Spearman rank correlations r		Vitamin D3	Ferritin	TIBC	EPO	Transferrin
Vitamin D3	Cor. Coefficient	**1.000**	0.064	-0.150	0.031	0.053
	Sig. (2-tailed)	-	0.372	0.069	0.774	0.551
Ferritin	Cor. Coefficient	0.064	**1.000**	0.092	-0.270	-0.021
	Sig. (2-tailed)	0.372	-	0.268	**0.010**	0.815
TIBC	Cor. Coefficient	-0.150	0.092	**1.000**	-0.044	-0.004
	Sig. (2-tailed)	0.069	0.268	-	0.685	0.965
EPO	Cor. Coefficient	0.031	-0.270	-0.044	**1.000**	0.044
	Sig. (2-tailed)	0.774	**0.010**	0.685	-	0.753
Transferrin	Cor. Coefficient	0.053	-0.021	-0.004	0.044	**1.000**
	Sig. (2-tailed)	0.551	0.815	0.965	0.753	-

3.4. Regression analysis of vitamin D3 and its confounders with ferritin in pregnant women

Pregnancy period, education level, and economic status were significantly associated with ferritin level ($p<0.05$). Vitamin D3 did not show a significant relationship with ferritin concentration. The overall regression model was statistically significant ($p=0.005$).

Table4. predict correlation of vitamin D3 and confounders with ferritin

Predictor	Unstandardized CoefficientB	*S.E.*	Standardized Coefficientsβ	t	P value	VIF	95% CI
Intercept	83.696	28.348		2.952	**0.004**		[27.768, 139.625]
Vitamin D3	0.055	0.149	0.026	0.366	0.714	1.095	[-0.239, 0.348]
Age	0.411	0.432	0.077	0.951	0.343	1.398	[-0.441, 1.263]
Gestational age	-1.132	0.534	-0.318	-2.118	**0.035**	4.767	[-2.186, -0.078]
BMI	-0.722	0.530	-0.098	-1.364	0.174	1.105	[-1.767, 0.323]
Education level	-3.021	1.277	-0.184	-2.366	**0.019**	1.280	[-5.541, -0.502]
Occupation	6.630	4.756	0.100	1.394	0.165	1.097	[-2.753, 16.012]
Physical activity	-2.087	4.793	-0.030	-0.436	0.664	1.031	[-11.543, 7.368]
Place of residence	-1.096	5.223	-0.015	-0.210	0.834	1.088	[-11.400, 9.208]
Economic status	-20.956	5.982	-0.244	-3.503	**0.001**	1.031	[-32.757, -9.154]
Trimester	11.931	6.980	0.252	1.709	0.089	4.604	[-1.841, 25.702]
Parital status	2.391	4.627	0.041	0.517	0.606	1.313	[-6.737, 11.519]

Dependent variable: ferritin. Model fit: $R^2 = 0.133$, adjusted $R^2 = 0.081$, $F = 2.566$, $P = 0.005$

Multiple Linear Regression Analysis of Predictors of Total Iron-Binding Capacity (TIBC) in Pregnant Women

Multiple linear regression analysis showed that serum vitamin D was not independently associated with total iron-binding capacity (TIBC) after adjustment for age, gestational age, BMI, education level, occupation, physical activity, place of residence, economic status, trimester, and marital status (β = -0.147, B = -0.251, 95% CI: -0.545 to 0.043, p = 0.094). None of the adjustment variables were statistically significant predictors of TIBC (all p > 0.05). The overall regression model was not statistically significant (F = 1.365, p = 0.196) and explained 9.9% of the variation in TIBC ($R^2 = 0.099$; adjusted $R^2 = 0.027$). Variance inflation factor (VIF) values ranged from 1.04 to 4.67, indicating no evidence of problematic multicollinearity.

Table5. Multiple Linear Regression of vitamin D and Adjustment Potential Confounders across TIBC

Predictor	Unstandardized Coefficient B	*S.E.*	Standardized Coefficients β	t	P value	VIF	95% CI
Intercept	314.156	28.211		11.136	0.000		[258.366, 369.946]
Vitamin D3	-0.251	0.149	-0.147	-1.686	0.094	1.151	[-0.545, 0.043]
Age	-0.830	0.459	-0.181	-1.806	0.073	1.509	[-1.738, 0.079]
Gestational age	-0.339	0.557	-0.107	-0.610	0.543	4.672	[-1.440, 0.761]
BMI	-0.026	0.527	-0.004	-0.050	0.960	1.083	[-1.069, 1.016]
Education level	-1.743	1.315	-0.124	-1.325	0.187	1.325	[-4.344, 0.858]
Occupation	6.715	4.777	0.119	1.406	0.162	1.089	[-2.731, 16.161]
Physical activity	-0.873	4.749	-0.015	-0.184	0.854	1.040	[-10.265, 8.520]
Place of residence	-6.140	5.216	-0.102	-1.177	0.241	1.136	[-16.455, 4.175]
Economic status	7.231	5.529	0.109	1.308	0.193	1.051	[-3.703, 18.166]
Trimester	10.067	7.040	0.246	1.430	0.155	4.457	[-3.854, 23.989]
Parital status	2.264	4.635	0.046	0.489	0.626	1.354	[-6.902, 11.431]

Dependent variable: TIBC. Model fit: $R^2 = 0.099$, adjusted $R^2 = 0.027$, $F = 1.365$, $P = 0.196$

3.5. Multiple Linear Regression Model Predicting Erythropoietin (EPO) Levels from Serum Vitamin D_3 and Potential Confounders

Multiple linear regression analysis showed that serum vitamin D was not independently associated with erythropoietin (EPO) levels after adjustment for age, gestational age, BMI, education level, occupation, physical activity, place of residence, economic status, trimester, and marital status (B = -0.047, β = -0.018, 95% CI: -0.633 to 0.538, p = 0.872). None of the independent variables were significantly associated with EPO levels (all p > 0.05), although physical activity showed a borderline association (B = 16.999, β = 0.207, p = 0.066). The overall regression model was statistically significant (F = 2.756, p = 0.002), explaining 14.1% of the variance in EPO levels ($R^2 = 0.141$; adjusted $R^2 = 0.090$). VIF values ranged from 1.12 to 5.28, indicating no evidence of problematic multicollinearity.

Table6. Multiple Linear Regression Analysis of Predictors of erythropoietin (EPO)

Predictor	Unstandardized Coefficient B	*S.E.*	Standardized Coefficients β	t	P value	VIF	95% CI
Intercept	-8.673	58.218		-0.149	0.882		[-124.601, 107.254]

Vitamin D3	-0.047	0.294	-0.018	-0.161	0.872	1.156	[-0.633, 0.538]
Age	-0.264	0.916	-0.039	-0.288	0.774	1.677	[-2.088, 1.560]
Gestational age	0.122	1.087	0.027	0.113	0.911	5.282	[-2.043, 2.288]
BMI	-0.743	0.978	-0.084	-0.760	0.450	1.128	[-2.689, 1.204]
Education level	2.885	2.613	0.148	1.104	0.273	1.639	[-2.319, 8.089]
Occupation	-8.497	9.366	-0.113	-0.907	0.367	1.404	[-27.147, 10.153]
Physical activity	16.999	9.107	0.207	1.866	0.066	1.120	[-1.136, 35.133]
Place of residence	-6.379	10.256	-0.071	-0.622	0.536	1.181	[-26.802, 14.044]
Economic status	8.946	12.708	0.078	0.704	0.484	1.121	[-16.359, 34.252]
Trimester	12.262	13.340	0.215	0.919	0.361	4.973	[-14.301, 38.825]
Parital status	5.686	8.714	0.078	0.653	0.516	1.316	[-11.665, 23.038]

Dependent variable: EPO. Model fit: $R^2 = 0.141$, adjusted $R^2 = 0.090$, $F = 2.756$, $P = 0.002$

3.6 Between Serum Vitamin D_3 and Transferrin After Adjustment for Potential Confounders

Multiple linear regression analysis demonstrated that serum vitamin D was not independently associated with transferrin levels after adjustment for age, gestational age, BMI, education level, occupation, physical activity, place of residence, economic status, trimester, and marital status (B = -0.007, β = -0.057, 95% CI: -0.031 to 0.017, p = 0.550). None of the potential confounding variables were significantly associated with transferrin levels (all $p > 0.05$), although BMI showed a borderline positive association (B = 0.082, β = 0.175, p = 0.069). The overall regression model was not statistically significant ($F = 0.619$, $p = 0.809$), explaining only 5.5% of the variance in transferrin levels ($R^2 = 0.055$; adjusted $R^2 = -0.034$). VIF values ranged from 1.05 to 4.41, indicating no evidence of problematic multicollinearity.

Table7. Multiple Linear Regression Analysis of Predictors of Transferrin

Predictor	Unstandardized Coefficient B	*S.E.*	Standardized Coefficients β	t	P value	VIF	95% CI
Intercept	1.702	2.511		0.678	0.499		[-3.271, 6.676]
Vitamin D3	-0.007	0.012	-0.057	-0.599	0.550	1.124	[-0.031, 0.017]
Age	-0.017	0.035	-0.053	-0.497	0.620	1.385	[-0.087, 0.052]
Gestational age	-0.010	0.045	-0.041	-0.215	0.830	4.412	[-0.099, 0.079]
BMI	0.082	0.045	0.175	1.833	0.069	1.130	[-0.007, 0.171]
Education level	0.039	0.105	0.037	0.372	0.710	1.252	[-0.169, 0.248]
Occupation	-0.495	0.455	-0.100	-1.088	0.279	1.057	[-1.396, 0.406]
Physical activity	-0.150	0.443	-0.031	-0.339	0.735	1.056	[-1.027, 0.727]
Place of residence	-0.112	0.411	-0.025	-0.272	0.786	1.077	[-0.927, 0.703]
Economic status	0.013	0.511	0.002	0.026	0.979	1.054	[-0.999, 1.025]
Trimester	-0.150	0.576	-0.048	-0.261	0.794	4.233	[-1.291, 0.990]
Parital status	-0.271	0.380	-0.072	-0.713	0.477	1.273	[-1.023, 0.481]

Dependent variable: transferrin. Model fit: $R^2 = 0.055$, adjusted $R^2 = -0.034$, F = 0.619, P = 0.809

3.7 Diagnostic Performance of Iron Biomarkers for Identifying Vitamin D adequacy in Pregnant Women

ROC analysis showed that ferritin (AUC = 0.538), TIBC (AUC = 0.452), EPO (AUC = 0.587), and transferrin (AUC = 0.487) all had poor diagnostic performance for identifying vitamin D adequacy (≥30 ng/mL). None of the AUCs were statistically significant (all $p > 0.05$), indicating that these biomarkers were not useful discriminators of vitamin D status in the study population. ROC analysis demonstrated that ferritin (AUC = 0.462), TIBC (AUC = 0.548), EPO (AUC = 0.413), and transferrin (AUC = 0.513) had poor diagnostic performance for identifying vitamin D Inadequacy (<30 ng/mL). None of the biomarkers showed statistically significant discriminatory ability (all $p > 0.05$), indicating limited value in predicting adequate vitamin D status among pregnant women.

Table8. Receiver Operating Characteristic (ROC) Diagnostic Metrics for Vitamin D Adequacy≥30 and Vitamin D InAdequacy<30 across ferritin, TIBC, EPO, and Transferrin (N = 196)

Clinical category	AUC	Std. Error	P value	95% CI
Vitamin D Adequacy≥30				
Ferritin	0.538	0.084	0.654	[0.374, 0.702]
TIBC	0.452	0.090	0.574	[0.276, 0.628]
EPO	0.587	0.085	0.313	[0.420, 0.753]
Transferrin	0.487	0.082	0.879	[0.326, 0.648]
Vitamin D Inadequacy <30				
Ferritin	0.462	0.084	0.654	[0.298, 0.626]
TIBC	0.548	0.090	0.574	[0.372, 0.724]
EPO	0.413	0.085	0.313	[0.247, 0.580]
Transferrin	0.513	0.082	0.879	[0.352, 0.674]

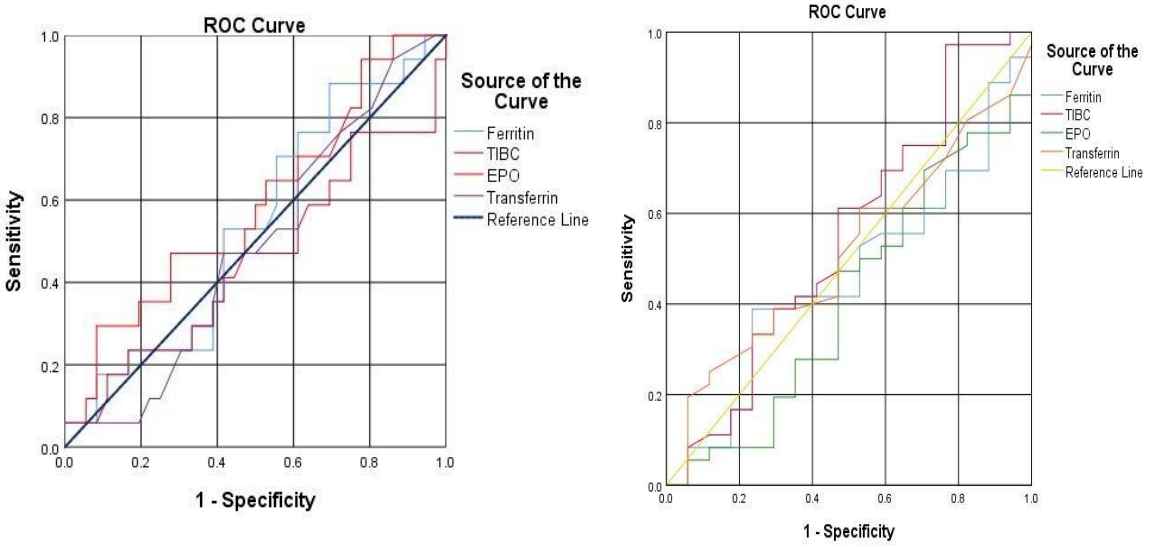

Figure 1. ROC Curves of Ferritin, TIBC, Erythropoietin, and Transferrin for Predicting Vitamin D inadequacy and Vitamin D inadequacy in Pregnancy

4. DISCUSSION

Research result show level education mother pregnant the highest ratio was above high school, totaling people 30.6%. High school education to on show level more knowledge and behavior good because education is one factor affecting formation behavior somebody. The result shows that most of the pregnant were overweight which is 53.1%. Educational efforts should be made to include safe vitamin D intake in antenatal care (Hitesh et al., 2023). The present study found showed vitamin D status was Inadequacy 25.1 ng/mL, agreed with A systematic literature review on the distribution of serum 25(OH)D among reproductive age Japanese women found that serum 25(OH)D level was especially low for perinatal women (Takaoka et al., 2017). Study from Indonesia with 203 women recruited showed that 195 women (96.06%) were suffering from hypovitaminosis D (Judistiani et al., 2019).

The mechanism of change vitamin D level during pregnancy may be explained by an increase of plasma volume of mother, placental function, vitamin D and calcium metabolism, and fetal growth. Expansion of plasma volume begins during the first week of pregnancy. It is known that plasma volume increases most steeply during the second trimester of pregnancy (de Haas et al., 2017).

The present study (table3) found no significant correlations between maternal serum vitamin D_3 levels and iron status biomarkers, including ferritin, TIBC, erythropoietin (EPO), and transferrin (all $p > 0.05$). Although vitamin D has been proposed to regulate iron metabolism by suppressing hepcidin and enhancing iron availability, the current findings suggest that vitamin D status was not independently associated with systemic iron status in this cohort of pregnant women. These results are consistent with previous studies reporting weak or non-significant associations between vitamin D and iron biomarkers during pregnancy and in healthy adults, indicating that physiological adaptations of pregnancy, iron supplementation, inflammation, and nutritional factors may exert a greater influence on iron homeostasis than vitamin D status alone (Smith and Tangpricha, 2015, Bacchetta et al., 2014, Ganz and Nemeth, 2015, Organization, 2023). Interestingly, ferritin showed a significant negative correlation with EPO ($r = -0.270$, $p = 0.010$), suggesting that reduced iron stores were associated with increased erythropoietin production, a well-recognized compensatory response to declining iron availability (O'Callaghan et al., 2023, Braithwaite et al., 2019).

Multiple linear regression analysis in **table4** showed serum vitamin D level was not positive associated with ferritin concentration ($\beta = 0.026$, $p = 0.714$), however overall $p=0.005$ (Munasinghe et al., 2019). Confounders like pregnancy period, educational level and economic status were significantly associated with ferritin concentration $p<0.05$. While other vitamin D Confounders were not significant predictor in the model $p>0.05$. Totally table showed vitamin D with its Confounders correlated with ferritin concentration ($p<0.05$), the regression model explained 13.3 % of the variation in ferritin concentration. Regression analysis in **table5** showed TIBC was not significant predictor in the model ($p>0.05$). Regression analysis in **table6** showed Serum vitamin EPO status was not significant predictor in the model ($p>0.05$). Disagreed with the study showed maternal 25(OH)D was inversely associated with erythropoietin at both midgestation ($P<0.05$) and delivery ($P <0.001$). Furthermore, vitamin D works synergistically with erythropoietin (EPO) in the maternal bone marrow; hypovitaminosis D blunts erythroid progenitor stimulation, compounding poor iron utilization and further dragging down circulating iron levels (Thomas et al., 2015).

Regression analysis in **table7** showed serum transferrin was not significant predictor in the model ($p>0.05$). disagreement In epidemiological and cross-sectional multivariate analyses, maternal Vitamin D levels display a significant inverse (negative) relationship with serum transferrin and Total Iron-Binding Capacity (TIBC) (Das et al., 2025, Wong et al., 2022).This occurs because transferrin increases as the body attempts to maximize its iron-binding and transport capacity during systemic or functional iron shortages.

5. CONCLUSIONS

Despite a high prevalence of maternal Vitamin D3 inadequacy (63.8%), serum Vitamin D3 levels do not correlate with or independently predict key iron regulatory biomarkers including ferritin, TIBC, EPO, and transferrin nor do these iron markers possess diagnostic utility for identifying Vitamin D status (AUC = 0.413–0.587, $p > 0.05$). Consequently, routine antenatal care must incorporate independent, dual-screening protocols for both micronutrients rather than relying on standard iron panels to infer Vitamin D status.

6. RECOMMENDATIONS

Further longitudinal and larger-scale studies are recommended to clarify the relationship between vitamin D_3 status and iron metabolism during pregnancy. Maternal factors such as gestational age and BMI should be considered in future analyses. Routine monitoring of vitamin D and iron status, along with appropriate nutritional management during pregnancy, is recommended to support maternal and fetal health. while future prospective studies incorporating additional regulators like hepcidin are needed to fully elucidate potential pathway interactions.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Ethics Statement

The ethics committee of Koya University's Faculty of Science and Health obtained institutional ethical clearance through an ethics form (010 Bio) on November 11, 2024.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent statement

Written informed consent was obtained from all participants before they filled out the questionnaires.

Consent for publication

Not applicable. No identifying personal or clinical details of participants are included in this manuscript**.**

Transparency statement

The lead author **Sayfaddin Sadraddin Hamad** affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Author Contributions

Sayfaddin Sadraddin Hamad: Conceptualization; data creation; methodology; writing—original draft; Visualization;; writing review & editing. **Esmail Salih Ibrahim Kakey**: Conceptualization; formal analysis; investigation; supervision methodology; project administration; supervision; writing review & editing.

REFERENCES

[1] BACCHETTA, J., ZARITSKY, J. J., SEA, J. L., CHUN, R. F., LISSE, T. S., ZAVALA, K., NAYAK, A., WESSELING-PERRY, K., WESTERMAN, M. & HOLLIS, B. W. 2014. Suppression of iron-regulatory hepcidin by vitamin D. *Journal of the American Society of Nephrology,* 25**,** 564-572.

[2] BENEDIK, E. 2022. Sources of vitamin D for humans. *Int J Vitam Nutr Res,* 92**,** 118-125.

[3] BLANCO-ROJO, R., PÉREZ-GRANADOS, A. M., TOXQUI, L., ZAZO, P., DE LA PIEDRA, C. & VAQUERO, M. P. 2013. Relationship between vitamin D deficiency, bone remodelling and iron status in iron-deficient young women consuming an iron-fortified food. *European journal of nutrition,* 52**,** 695-703.

[4] BRAITHWAITE, V. S., CROZIER, S. R., D'ANGELO, S., PRENTICE, A., COOPER, C., HARVEY, N. C. & JONES, K. S. 2019. The Effect of Vitamin D Supplementation on Hepcidin, Iron Status, and Inflammation in Pregnant Women in the United Kingdom. *Nutrients,* 11.

[5] DAS, A., BAI, C.-H., CHANG, J.-S., HUANG, Y.-L., WANG, F.-F., HSU, C.-Y., CHEN, Y.-C. & CHAO, J. C. 2025. A ferritin-related dietary pattern is positively associated with iron status but negatively associated with vitamin D status in pregnant women: a cross-sectional study. *European Journal of Nutrition,* 64**,** 30.

[6] DE HAAS, S., GHOSSEIN-DOHA, C., VAN KUIJK, S. M., VAN DRONGELEN, J. & SPAANDERMAN, M. 2017. Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound in Obstetrics & Gynecology,* 49**,** 177-187.

[7] GANZ, T. & NEMETH, E. 2015. Iron homeostasis in host defence and inflammation. *Nature Reviews Immunology,* 15**,** 500-510.

[8] HITESH, T., KHATUJA, R., AGRAWAL, P., DHAMNETIYA, D., JHA, R. P. & RENJHEN, P. 2023. Unlocking the mystery of the role of Vitamin D in iron deficiency anemia in antenatal women: a case control study in a tertiary care hospital in New Delhi. *BMC Pregnancy and Childbirth,* 23**,** 749.

[9] HOLICK, M. F., BINKLEY, N. C., BISCHOFF-FERRARI, H. A., GORDON, C. M., HANLEY, D. A., HEANEY, R. P., MURAD, M. H. & WEAVER, C. M. 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab,* 96**,** 1911-30.

[10] HOLLIS, B. W., JOHNSON, D., HULSEY, T. C., EBELING, M. & WAGNER, C. L. 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res,* 26**,** 2341-57.

[11] JARMUND, A. H., GISKEØDEGÅRD, G. F., RYSSDAL, M., STEINKJER, B., STOKKELAND, L. M. T., MADSSEN, T. S., STAFNE, S. N., STRIDSKLEV, S., MOHOLDT, T. & HEIMSTAD, R. 2021. Cytokine patterns in maternal serum from first trimester to term and beyond. *Frontiers in immunology,* 12**,** 752660.

[12] JUDISTIANI, R. T. D., MADJID, T. H., IRIANTI, S., NATALIA, Y. A., INDRATI, A. R., GHOZALI, M., SRIBUDIANI, Y., YUNIATI, T., ABDULAH, R. & SETIABUDIAWAN, B. 2019. Association of first trimester maternal vitamin D, ferritin and hemoglobin level with third trimester fetal biometry: result from cohort study on vitamin D status and its impact during pregnancy and childhood in Indonesia. *BMC pregnancy and childbirth,* 19**,** 112.

[13] JUDISTIANI, R. T. D., SAMOSIR, S. M., IRIANTI, S., PURWARA, B. H., SETIABUDIAWAN, B., MOSE, J. C. & HANDONO, B. 2020. Correlation of maternal serum hepcidin, soluble transferrin receptor (sTfR) and cholecalciferol with third trimester anemia: findings from a nested case-control study on a pregnancy cohort. *The Indonesian Biomedical Journal,* 12**,** 361-7.

[14] KALUDJEROVIC, J. & VIETH, R. 2010. Relationship between vitamin D during perinatal development and health. *Journal of midwifery & women's health,* 55**,** 550-560.

[15] MULLIGAN, M. L., FELTON, S. K., RIEK, A. E. & BERNAL-MIZRACHI, C. 2010. Implications of vitamin D deficiency in pregnancy and lactation. *Am J Obstet Gynecol,* 202**,** 429.e1-9.

[16] MUNASINGHE, L. L., EKWARU, J. P., MASTROENI, M. F., MASTROENI, S. S. & VEUGELERS, P. J. 2019. The association of serum 25-hydroxyvitamin D concentrations with elevated serum ferritin levels in normal weight, overweight and obese Canadians. *PLoS One,* 14**,** e0213260.

[17] O'CALLAGHAN, K. M., QAMAR, H., GERNAND, A. D., ONOYOVWI, A., ZLOTKIN, S., MAHMUD, A. A., AHMED, T., KEYA, F. K. & ROTH, D. E. 2023. Maternal prenatal, with or without postpartum, vitamin D3 supplementation does not improve maternal iron status at delivery or infant iron status at 6 months of age: secondary analysis of a randomised controlled trial. *BMJ Nutrition, Prevention & Health,* 6**,** 282.

[18] O'CALLAGHAN, K., TARIQ, U., GERNAND, A., TINAJERO, M., ONOYOVWI, A., ZLOTKIN, S., AL MAHMUD, A., AHMED, T., KEYA, F. & ROTH, D. 2020. Effect of Maternal Vitamin D Supplementation on Iron Status During Pregnancy. *Current Developments in Nutrition,* 4**,** nzaa054_126.

[19] OMAR, S. Y., AWLA, A. H., SALIH, M. I. & HAMADAMIN, B. J. 2025. Impact of Vitamin D Deficiency on Iron Deficiency Anemia: A Comparative Analysis in Iraqi Population. *ARO-THE SCIENTIFIC JOURNAL OF KOYA UNIVERSITY,* 13**,** 108-113.

[20] ORGANIZATION, W. H. 2023. *WHO recommendations on antenatal care for a positive pregnancy experience: screening, diagnosis and treatment of tuberculosis disease in pregnant women. Evidence-to-action brief: Highlights and key messages from the World Health Organization's 2016 global recommendations*, World Health Organization.

[21] PILZ, S., MÄRZ, W., CASHMAN, K. D., KIELY, M. E., WHITING, S. J., HOLICK, M. F., GRANT, W. B., PLUDOWSKI, P., HILIGSMANN, M. & TRUMMER, C. 2018. Rationale and plan for vitamin D food fortification: a review and guidance paper. *Frontiers in endocrinology,* 9**,** 373.

[22] ROSELAND, J. M., PHILLIPS, K. M., PATTERSON, K. Y., PEHRSSON, P. R. & TAYLOR, C. L. 2018. Vitamin D in foods: An evolution of knowledge. *Vitamin D*. Elsevier.

[23] SMITH, E. M., ALVAREZ, J. A., KEARNS, M. D., HAO, L., SLOAN, J. H., KONRAD, R. J., ZIEGLER, T. R., ZUGHAIER, S. M. & TANGPRICHA, V. 2017. High-dose vitamin D(3) reduces circulating hepcidin concentrations: A pilot, randomized, double-blind, placebo-controlled trial in healthy adults. *Clin Nutr,* 36**,** 980-985.

[24] SMITH, E. M. & TANGPRICHA, V. 2015. Vitamin D and anemia: insights into an emerging association. *Current Opinion in Endocrinology, Diabetes and Obesity,* 22**,** 432-438.

[25] TAKAOKA, N., NAGAO, M., UMEZAWA, M., SAIRENCHI, T., HARUYAMA, Y. & KOBASHI, G. 2017. Distribution of serum 25-hydroxyvitamin D levels of reproductive age Japanese women. *[Nihon Koshu Eisei Zasshi] Japanese Journal of Public Health,* 64**,** 133-142.

[26] THOMAS, C. E., GUILLET, R., QUEENAN, R. A., COOPER, E. M., KENT, T. R., PRESSMAN, E. K., VERMEYLEN, F. M., ROBERSON, M. S. & O'BRIEN, K. O. 2015. Vitamin D status is inversely associated with anemia and serum erythropoietin during pregnancy. *The American Journal of Clinical Nutrition,* 102**,** 1088-1095.

[27] VAN DEN BROEK, N. R. & LETSKY, E. A. 2000. Etiology of anemia in pregnancy in south Malawi. *The American journal of clinical nutrition,* 72**,** 247S-256S.

[28] WHO, U. 2014. Global nutrition targets 2025: breastfeeding policy brief (WHO/NMH/NHD14. 7). *Geneva: World Health Organization*.

[29] WONG, R. S., TUNG, K. T., CHAN, Y. W., CHAN, B. N., LEUNG, W.-C., YAM, J. C. & IP, P. 2022. Adequate dietary intake and vitamin D supplementation: A study of their relative importance in determining serum vitamin D and ferritin concentrations during pregnancy. *Nutrients,* 14**,** 3083.