

Predict correlation maternal plasma vitamin D3 levels with some thyroid function biomarkers (TSH, TT4, and TT3) during pregnancy in Koya district, Erbil city, Iraq

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Abstract

Background: Vitamin D deficiency and insufficiency (serum vitamin D <30 ng/ml) and thyroid dysfunctions are prevalent issues globally, particularly during pregnancy. Evidence suggests a potential link between thyroid function and vitamin D levels. This study aims to investigate the correlation between serum vitamin D levels and thyroid functions (thyroid-stimulating hormone (TSH), total Triiodothyronine (TT3), and total Thyroxine (TT4) in women across all trimesters of pregnancy. **Objective:** predict correlation of vitamin D3 and some thyroid function hormones during pregnancy.

Methods: Observational, analytical, cross-sectional study was conducted among pregnant women attending primary healthcare centers in Koya district, Erbil city. The sample group consisted of all trimester pregnant women who attended routine pregnancy check-ups and had complete vitamin D levels and thyroid function tests (n=196). The sociodemographic data form and blood serum level assessment forms were used as data collection tools. Immunoassay techniques were to measure parameters MAGLUMIX8. The data were analyzed using descriptive statistics and correlation analysis, multiple linear regression analysis were performed to estimate relationships between vitamin D3 with adjustment for potential confounders (BMI, trimester, education, etc.). Binary Logistic Regression OR is used to predict the probability of dichotomous thyroid parameters.

Results: A multiple linear regression was conducted to predict [vitamin D3 with adjusted confounders] based on [TSH], [TT4], and [TT3]. The predictors explained a non-significant proportion of variance in [vitamin D3], $R^2 = 0.095$; Adjusted $R^2 = -0.022$, $F = 1.305$; $P = 0.228$. Standardized coefficients indicated that [TSH] had the strongest positive association with the outcome ($\beta = -0.097$, $p > 0.05$), followed by [TT4] $R^2 = 0.052$; Adjusted $R^2 = -0.024$, $F = 0.679$; $P = 0.757$. Whereas [TT3] did not contribute significantly to the model $R^2 = 0.061$; Adjusted $R^2 = -0.013$, $F = 0.824$; $P = 0.616$. Vitamin D3 was not a significant predictor for any of the thyroid parameters ($p > 0.05$). Among all investigated maternal confounders, only BMI demonstrated a significant positive association with TSH levels ($\beta = 0.214$, $t = 2.50$, $p = 0.014$). All other confounders, including age, trimester, and pregnancy period, failed to significantly predict thyroid hormone levels. Logistic regression (OR) was used to assess the severity of vitamin D3 on thyroid work.

Conclusion: The correlation analysis revealed no significant relationship between the thyroid function tests and vitamin D levels of the pregnant women. It was determined that vitamin D levels do not affect thyroid gland function across all

Keywords: Vitamin D3, TSH, TT4, TT3.

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Introduction

Pregnancy enhances the activation and production of steroid hormones, with the breakdown of thyroxine-binding globulin (TBG), resulting in increased levels of TBG, total tetraiodothyronine (TT4), and total

Triiodothyronine (TT3) (Rahman et al., 2007). As a result, thyroid dysfunction is among the most prevalent endocrine disorders impacting women of reproductive age. Thyroid disorders not only impact fertility and pregnancy outcomes but may potentially affect the development of the infant nervous system

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and elevate the occurrence of congenital problems. Pregnancy hyperthyroidism occurs in approximately 0.2% of cases, whereas hypothyroidism is more prevalent at 2.5% (Zdravetska and Kocova, 2021). It is acknowledged that the assessment of thyroid functions varies by trimester (Springer et al., 2017). Several hormonal and metabolic changes during each trimester lead to complicated influences on maternal thyroid physiology (Bohn and Adeli, 2022). Women of reproductive age have an elevated incidence of thyroid disorders, including chronic thyroiditis, thyroid dysfunctions, Hashimoto's hypothyroidism, and Graves' disorder, necessitating an analysis of factors influencing thyroid function tests. Thyroid hormone receptors and vitamin D receptors are identical steroid hormone receptors involved in the production of various genes, and any alterations in these structural genes increase the susceptibility to autoimmune disorders, that involve autoimmune diseases of the thyroid like Hashimoto's and Graves' disease (Zhao et al., 2021). Vitamin D concentrations have been found to be diminished in instances of autoimmune thyroiditis; Vitamin D insufficiency is increasing, and vitamin D intake can reduce thyroid antibody levels and decrease autoantibody levels with treatment (temd.org.tr). Vitamin D deficiency is widespread globally, about one billion individuals in developing nations are facing this lack of supply (Babić Leko et al., 2023). Vitamin D insufficiency is more prevalent in Asian nations and Middle Eastern countries than in other regions; in Turkey, the prevalence is about 49%, which is comparable to that of other Middle Eastern nations (Jiang et al., 2023). Vitamin D deficiency throughout pregnancy is prevalent among high-risk populations, including vegetarians, individuals with limited sun exposure, and those with darker skin colours. Vitamin D3 is a fat-soluble vitamin and a steroid hormone precursor, which is primarily synthesized in the skin after exposure to ultraviolet B radiation. The liver is the first organ where hydroxylation occurs to produce 25 - hydroxyvitamin D (25(OH) D). Further hydroxylation continues mainly in the kidneys to produce the active form 1, 25 - dihydroxy vitamin D (1, 25(OH) 2D), besides the placenta and several other target organs (Holick, 2014). There are two major types of vitamin D3, a steroid: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (Omar et al., 2025). Skin synthesizes approximately 80% of vitamin D3 via UVB radiation (295–315nm), 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). In fact, less than 10% of vitamin D is derived from main dietary sources such as dairy, eggs, fish

and meat (Green et al., 2015, Liu et al., 2017). Moreover, vitamin D has relatively durable stability over several weeks, and approximately 99% of circulating 25(OH) D3 is bound to vitamin D-binding proteins. Interestingly, its gradual oscillation depends on solar radiation rather than on vitamin D precursors that are gained from food items (Vondra et al., 2015). Vitamin D deficiency is being identified more and more as a common nutritional and endocrine disorder globally, specifically affecting women of reproductive age as well as pregnant women. During pregnancy, changes in the body like expanded blood plasma volume, different hormone metabolism, and elevated nutritional needs may lead to vitamin D insufficiency even more. part from the well-known pivotal role for calcium and bone metabolism, vitamin D has steroid hormone functions which influence endocrine pathways, including the thyroid physiology interactions (Vitamin, 2007, Bouillon et al., 2019). Thyroid gland functioning normally plays an important role in keeping the metabolic balance of a mother and in providing nutrients to the fetus for its growth and brain development. The need is even more especially in the early stages of pregnancy when the fetus completely depends on the mother's thyroid hormones. Thyroid function markers such as thyroid-stimulating hormone (TSH), total Triiodothyronine (TT3), and total Thyroxine (TT4) demonstrate physiological changes during pregnancy as a result of stimulation by human chorionic gonadotropin, and estrogen-induced increments in thyroid-binding globulin (Glinioer, 2004, Alexander et al., 2017). Even small changes or subclinical deviations in these parameters have been linked to pregnancy complications such as miscarriage, gestational hypertension, preterm delivery, and offspring neurocognitive development impairment (Korevaar, 2017 #129). Latest information shows that vitamin D might be biologically connected to thyroid functions. VDR along with vitamin D metabolizing enzymes are found in thyroid tissue and immune cells, thus vitamin D might be involved with thyroid hormone production, release, and peripheral (Muscogiuri et al., 2015). Many observational studies have demonstrated negative correlations of serum vitamin D levels with TSH and positive associations with TT3 and TT4 in pregnant women; on the other hand, the findings of the studies are not consistent because of variation in study design, gestational age, and population characteristics (Mackawy et al., 2013, Zubyra et al., 2022, Zhou et al., 2020, Kim, 2016). Vitamin D deficiency as well as thyroid dysfunction issues during pregnancy is very common. Thus, it is quite important from the clinical and public health points of view to investigate how serum vitamin D and thyroid function biomarkers (TSH, TT3, and TT4) are correlated. Understanding this relationship

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more deeply may help with early identification of endocrine disorders. Also, this knowledge might be useful for the development of prevention and treatment methods that would result in better health outcomes for mothers and fetuses (Mackawy et al., 2013, Chaudhary et al., 2016, Farias et al., 2020). Understanding these associations could help in early detection of pregnant women who are at risk and thus direct nutritional interventions for better maternal and fetal health outcomes. In light of these results, the aim of this research is to add to the knowledge by examining the association between vitamin D levels and thyroid functions in pregnant women during pregnancy.

Research Problem Statement

During pregnancy, vitamin D is very important for regulating the endocrine system. Nevertheless, information concerning the correlation between vitamin D levels, thyroid function indicators in pregnant women is still insufficient. Subtle alterations in thyroid function biomarkers thyroid stimulating hormone (TSH), total Triiodothyronine (TT3), and total Thyroxine (TT4) have been associated with adverse pregnancy outcomes. However, the extent to which vitamin D status influences these parameters during pregnancy remains unclear.

Thus, the problem of research in the study can be summarized as the absence of comprehensive evidence explaining the relationship between serum levels of vitamin D, thyroid function indices, as it has to be determined if the deficiency of vitamin D can cause thyroid dysfunction during pregnancy.

Research Gap

Despite the growing body of evidence that associates the potential involvement of vitamin D in the regulation of the endocrine, the holistic interaction of the status of vitamin D, the levels of thyroid function in pregnancy remains relatively unknown in one condition.

Primary Research Question

How is maternal serum vitamin D concentration related to biomarkers of thyroid function (TSH, TT3, and TT4)? Mechanistic and Pathophysiological Questions, Does vitamin D deficiency contribute to altered thyroid hormone regulation in pregnant women? Should vitamin D screening be integrated into routine antenatal care for early identification of thyroid abnormalities?

Hypothesis:

H1: hypothesizes no statistically significant association between vitamin D3 levels with certain thyroid function indices (TSH, TT3, and TT4) during pregnancy.

H0: hypothesizes statistically significant association between vitamin D3 levels with certain thyroid function indices (TSH, TT3, and TT4) during pregnancy.

Materials and Methods

This is an Observational, Analytical; Cross-Sectional Study entitled: Predict correlation maternal plasma vitamin D3 levels with some thyroid function biomarkers (TSH, TT4, and TT3) during pregnancy in Koya district, Erbil city, Iraq. This study was conducted retrospectively using data from 196 pregnant women who visited the three MCH centers in Koya district and Shahid dr.Khalid Hospital, obstetric unit clinic for regular pregnancy check-up from June 2024 to January 2025. Permissions for the study were obtained from the Koya University. 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. The study population consisted of 211 pregnant women who attended the clinic for pregnancy check-ups. The sample group included pregnant women in across all trimesters that had both thyroids function tests, measured vitamin D levels, and had no known pre-existing conditions before pregnancy. The data collection tools used in this study included a sociodemographic data form and a blood serum levels form (for thyroid function tests and vitamin D levels). The sociodemographic data form consisted of questions regarding the woman's age, weight, height, Body Mass Index (BMI), educational level, gestational week, gravida. The calculation of the gestational week was based on the last menstrual period (LMP). If the woman did not know her LMP, the gestational week was determined by comparing ultrasound reports. The Blood Serum Levels Form included the pregnant women's vitamin D levels and thyroid function tests (TSH, TT3, TT4) during pregnancy. Before data collection began, the necessary legal permissions were obtained, and it was ensured that data collection adhered to the Personal Data Protection Law, with a commitment that the data would be used solely for scientific purposes. Blood samples obtained from pregnant women during their prenatal visit (all trimesters) were evaluated. To determine the relationship between

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vitamin D and TT4, TT3, TSH, and 25(OH)D were analyzed used Immuno assay automated analyzer (MAGLUMI X8, China). Blood samples were taken from all pregnant women after at least 8 hours of fasting. The collected blood samples were transferred to the MCH laboratory then aliquoted and stored in ultra-freezer (-20C) until the tests will be run. The blood samples were centrifuged to obtain serum and stored at 4°C. TT3, TT4, and TSH levels were measured by the quantitative analysis of vitamin D was conducted using the Snibe (Maglumi X8, China) competitive immune assay technique, following a standard curve reviewed by two qualified reviewers according procedures. Endocrinology According to Society standard to the clinical Iraqi Practice Guidelines, vitamin D levels of 30 ng/ml and above are defined as normal/adequate, levels between <30 ng/ml as vitamin D insufficiency/deficiency. All were excluded non pregnant women and those had previously chronic disease as diabetes, hypertension, and if blood sample hemolysis. Fifteen patients with chronic inflammation diseases were not included. 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 adaptations. Alpha (α) = 0.05. Statistical Power (1 - β) = 0.99. SPSS software statistics models, version 25.0, module, was employed to carry out all data cleaning and analysis. Descriptive statistics, such as mean, standard deviation have been utilized to explain the nature of data. The multiple linear regression analysis test used to estimate the associations between serum vitamin D3 concentrations with thyroid function markers independently. Scatter plot chart builder was used to draw linear graphs and to show correlation between vitamin D3 as dependent and percept independent variables included TSH, TT4, and TT3 The interactions between the status of vitamin D severity and thyroid disorder risk were performed using binary logistic analysis (Odds ratios). P-values less than 0.05 were considered statistically significant correlation in all tests.

3. Results

3.1. Socio-demographic and information about study sample

Table1. Vitamin D inadequacy (<30 ng/mL) was observed in 63.8 % of participants, This suggests that Vitamin D deficiency was common among the studied pregnant women. More than half (59.2%) participants were with in the second trimester. Highest educational level group (30.6%) was above high school. Regarding BMI status more than (53.1%) was overweight. Primigravida women accounted for 51.5% of the participants, while multigravida women represented 48.5%. This indicates an almost equal distribution between first-time and multiparous pregnancies. Most (66.83%) of the pregnant was within age 21 to 30 years.

Table1. Socio-demographic and Obstetric Characteristics of the Study Participants (N = 196)

Variable s	Categories	Frequen cy N	Percenta ge %
Vitamin D3 status	Insufficient/deficient <30	125	63.8
	Sufficient >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	60	30.6
BMI status	Under weight	1	0.5
	Normal weight	48	24.5
	Over weight.	104	53.1
Parital state	Obese	43	21.9
	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	40	20.4

3.2. Descriptive statistics

Table2. Mean and Standard Deviation of Thyroid Biomarkers TSH, TT4, and TT3 during pregnancy (N = 196)

variabl e	Mean± S.D.	Standar d error	Skewne ss	Kurtosi s
TSH	2.000±1.363	0.109	1.839	5.224
TT4	8.033±2.409	0.197	0.423	3.983
TT3	1.936±0.51	0.045	-0.655	1.382

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3.3. Multiple Linear Regression of vitamin D3 and its confounders with TSH

A multiple linear regression analysis was performed to evaluate whether maternal Vitamin D3 levels and various socio-demographic and clinical confounding factors could significantly predict Thyroid-Stimulating Hormone (TSH) levels (Table 3). The overall regression model was not statistically significant, indicating a poor fit for the data, ($df=1$), $F= 0.791$, $p = 0.649$. The model accounted for only 6.0% of the total variance in TSH levels ($R^2= 0.060$, Adjusted $R^2= -0.016$). Crucially, maternal Vitamin D3 did not emerge as a significant independent predictor of TSH concentrations ($\beta = -0.102$, $t = -1.148$, $p = 0.253$, 95% CI [-0.026, 0.007]). Similarly, none of the evaluated confounding variables including maternal age ($p = 0.353$), body mass index (BMI) ($p = 0.342$), pregnancy period ($p = 0.631$), or trimester ($p = 0.793$) demonstrated a statistically significant association with TSH levels. Multicollinearity was not a concern for the majority of the predictors ($VIF < 2$), though expectedly higher VIF values were observed between highly related gestational variables, namely pregnancy period ($VIF = 5.938$) and trimester ($VIF = 5.758$).

Table3. predicts correlation of vitamin D3 with confounders and TSH.

Predictor	Unstandardized Coefficient B	Standardized Coefficient β	P value	VIF	95% CI
Constant	3.403	1.654	2.058	0.042	[0.133, 6.673]
Vitamin D3	-0.009	0.008	-0.102	-1.148	1.153 44 0.026, 0.007]
Age	-0.024	0.025	-0.093	-0.932	0.353 46 0.074, 0.026]
Pregnancy period	-0.016	0.033	-0.097	-0.681	5.938 38 0.081, 0.049]

BMI	0.028	0.029	0.083	0.953	0.342	1.108	[-0.036, 0.086]
Education level	-0.020	0.071	-0.027	-0.286	0.776	1.285	[-0.161, 0.121]
Occupation	-0.334	0.267	-0.109	-1.251	0.213	1.107	[-0.862, 0.194]
Physical activity	0.287	0.275	0.088	1.041	0.300	1.038	[-0.258, 0.831]
Place of residence	-0.304	0.289	-0.092	-1.050	0.295	1.113	[-0.876, 0.268]
Economic status	-0.382	0.336	-0.097	-1.137	0.257	1.068	[-1.046, 0.282]
Trimester	0.113	0.228	0.052	0.263	0.793	5.758	[-0.733, 0.958]
Parital status	-0.078	0.262	-0.029	-0.299	0.766	1.357	[-0.597, 0.440]

Dependent variable: TSH, $R^2 = 0.060$; Adjusted $R^2 = -0.016$, $F = 0.791$; $P = 0.649$

3.4. Multiple Linear Regression of vitamin D3 and its confounders with TT4

A multiple linear regression analysis was executed to evaluate the predictive capacity of maternal plasma Vitamin D3 and selected socio-demographic and clinical confounding variables on Total Thyroxine (TT4) concentrations (Table 4).

The overall regression model was statistically non-significant and demonstrated a poor fit for the empirical data, $df=1$, $F = 0.710$, $p = 0.727$. The full set of independent variables accounted for only 5.4% of the total variance in maternal TT4 values, with the

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adjusted model reflecting the lack of explanatory power over chance ($R^2 = 0.054$, Adjusted $R^2 = -0.022$).

Critically, maternal Vitamin D3 did not serve as a significant independent predictor of TT4 levels, displaying an approximately flat relationship ($\beta = 0.015$, $t = 0.177$, $p = 0.859$, 95% CI [-0.025, 0.030]). Moreover, none of the included confounding parameters reached the threshold for statistical significance. Clinical variables such as maternal age ($\beta = -0.153$, $p = 0.130$), BMI ($\beta = -0.075$, $p = 0.389$), and gestational progression metrics including pregnancy period ($p = 0.951$) and trimester ($p = 0.951$) yielded no distinct influence on TT4 outcomes. Similarly, lifestyle and socio-demographic variables, including physical activity ($p = 0.103$) and education level ($p = 0.110$), did not meaningfully alter the dependent variable.

Collinearity diagnostics revealed that multicollinearity was absent across the majority of the predictors ($VIF < 2$). However, structurally anticipated high Variance Inflation Factors were observed between the interconnected gestational parameters: pregnancy period ($VIF = 5.710$) and trimester ($VIF = 5.521$).

Table4. Predict correlation of vitamin D3 with confounders and TT4

Predictor	Unstandardized Coefficient B	Standardized Coefficient β	P value	VIF	95% CI
Constant	9.339	2.854	3.273	0.01	[3.696, 14.982]
Vitamin D3	0.002	0.014	0.015	0.177	[0.859, 0.025]
Age	-0.065	0.043	-0.153	-0.1	[0.859, 0.025]
Pregnancy period	-0.004	0.060	-0.012	-0.9	[0.859, 0.025]

BMI	-0.045	0.052	-0.075	-0.3	0.889	1.082	[-0.149, 0.058]
Education level	-0.198	0.123	-0.151	-0.1	1.610	1.280	[-0.442, 0.045]
Occupation	-0.052	0.496	-0.009	-0.9	0.117	1.134	[-1.033, 0.930]
Physical activity	0.804	0.490	0.139	1.639	0.103	1.046	[-1.773, 1.072]
Place of residence	-0.080	0.502	-0.014	-0.8	0.174	1.091	[-0.913, 1.260]
Economic status	-0.121	0.576	-0.018	-0.8	0.234	1.044	[-1.018, 1.558]
Trimester	-0.047	0.764	-0.012	-0.9	0.051	5.521	[-1.464, 1.380]
Parital status	0.464	0.463	0.097	1.0	0.318	1.346	[-0.452, 1.380]

Dependent variable: TT4, $R^2 = 0.054$; Adjusted $R^2 = -0.022$, $F = 0.710$; $P = 0.727$

3.5. Multiple Linear Regression of vitamin D3 and its confounders with TT3

A final multiple linear regression models were constructed to assess the impact of maternal plasma Vitamin D3 concentrations and selected socio-demographic and clinical confounding factors on Total Triiodothyronine (TT3) levels (Table 5). The global regression model did not achieve statistical significance and demonstrated low predictive capability, $df = 1$, $F = 0.851$, $p = 0.590$. Collectively, the independent variables explained 6.3% of the variance observed in maternal TT3 concentrations, with the adjusted model reinforcing the lack of explicit explanatory strength ($R^2 = 0.063$, Adjusted R^2

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= -0.011). Maternal Vitamin D3 status did not function as a statistically significant independent predictor of TT3 concentrations ($\beta = 0.022$, $t = 0.251$, $p = 0.802$, 95% CI [-0.005, 0.007]). Furthermore, no clinical or socio-demographic confounders significantly influenced TT3 levels within this model. Maternal age ($p = 0.438$), BMI ($p = 0.265$), and gestational progress indicators—including pregnancy period ($\beta = -0.210$, $p = 0.272$) and trimester ($\beta = 0.208$, $p = 0.264$)—failed to demonstrate significant associations. While maternal education level showed a minor trend toward an inverse relationship with TT3 levels, it did not cross the threshold for statistical significance ($\beta = -0.162$, $t = -1.755$, $p = 0.081$). Other lifestyle indicators, such as physical activity ($p = 0.868$) and occupation ($p = 0.644$), remained statistically inert. Consistent with the diagnostic profiles of the previous models, multicollinearity was negligible for the majority of the independent covariates ($VIF < 2$), except for the expectedly coupled gestational variables of pregnancy period ($VIF = 5.420$) and trimester ($VIF = 5.134$).

Table 5. Predict correlation of vitamin D3 with confounders and TT3

Predictor	Unstandardized Coefficient B	Standardized Coefficient β	P value	VIF	95% CI
Constant	2.439	0.616	3.956	0.000	[1.220, 3.657]
Vitamin D3	0.001	0.003	0.022	0.251	[-0.005, 0.007]
Age	-0.007	0.009	-0.075	0.738	[-0.013, 0.001]
Pregnancy period	-0.014	0.012	-0.210	1.103	[-0.028, 0.001]
BMI	0.012	0.011	0.096	1.119	[-0.003, 0.027]

Education level	-0.046	0.026	-0.162	-1.755	0.081	[-0.306, 0.218]
Occupation	0.048	0.03	0.040	0.462	0.644	[-0.115, 0.227]
Physical activity	-0.017	0.099	-0.014	-0.166	0.868	[-0.103, 0.071]
Place of residence	-0.025	0.08	-0.020	-0.232	0.819	[-0.111, 0.061]
Economic status	-0.106	0.028	-0.070	-0.832	0.409	[-0.256, 0.086]
Trimester	0.173	0.054	0.208	1.122	0.264	[-0.047, 0.478]
Parital status	-0.143	0.096	-0.139	-1.480	0.138	[-0.378, 0.096]

Dependent variable: TT3, $R^2 = 0.063$; Adjusted $R^2 = -0.011$, $F = 0.851$; $P = 0.590$

3.6. Logistic regression of thyroid predictors with plasma vitamin d3 levels

A multivariate binary logistic regression analysis was conducted to determine whether clinical classifications of maternal thyroid parameters act as independent predictors of systemic Vitamin 3 status. The independent predictor variables, Thyroid-Stimulating Hormone (TSH), Total Thyroxine (TT4), and Total Triiodothyronine (TT3) were categorized into "Normal" and "Abnormal" cohorts based on standard maternal serum reference thresholds.

The logistic regression model revealed no statistically significant predictive relationships between maternal thyroid categories and Vitamin D3 status. Serum TSH levels within the normal range ($0.1 \leq \text{TSH} \leq 4.0$ mIU/mL) did not significantly alter the likelihood of the outcome relative to the abnormal reference group ($B = -0.020$, Wald = 1.013, $p = 0.314$, OR = 0.980,

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95% CI [0.942, 1.020]). Similarly, variations in maternal circulating peripheral thyroid hormones did not yield significant predictive value. Euthyroid baseline states for TT4 ($7.0 \leq \text{TT4} \leq 15.0 \mu\text{g/dL}$) showed a completely flat correlation with the dependent variable ($B = -0.007$, Wald = 0.346, $p = 0.557$, OR = 0.994, 95% CI [0.972, 1.015]). Finally, maternal TT3 concentrations within standard clinical reference ranges ($1.2 \leq \text{TT3} \leq 2.8 \text{ ng/mL}$) failed to function as an independent biomarker for predicting plasma Vitamin D3 shifts ($B = -0.003$, Wald = 0.029, $p = 0.864$, OR = 0.997, 95% CI [0.962, 1.033]). Taken together, these multivariate statistics indicate that classifying maternal thyroid markers as binary normal or abnormal categories does not supply significant explanatory insight regarding maternal Vitamin D3 levels in this dataset.

Table6. Multivariate Binary Logistic Regression of thyroid predictor outcomes [TSH, TT4, and TT3] with Maternal Plasma Vitamin D3

Predictor	Categories	B	S.E.	Wald	p value	OR	95% CI
TSH	Normal ($0.1 \leq \text{TSH} \leq 4 \mu\text{IU/mL}$)	-0.0	0.0	1.0	0.3	0.9	[0.942, 1.020]
	Abnormal ($0.1 \geq \text{TSH} \geq 4 \mu\text{IU/mL}$)	20	20	13	14	80	
TT4	Normal ($7 \leq \text{TT4} \leq 15 \mu\text{g/dL}$)	-0.0	0.0	0.3	0.5	0.9	[0.972, 1.015]
	Abnormal ($7 \geq \text{TT4} \geq 15 \mu\text{g/dL}$)	07	11	46	57	94	
TT3	Normal ($1.2 \leq \text{TT3} \leq 2.8 \text{ ng/mL}$)	-0.0	0.0	0.0	0.8	0.9	[0.962, 1.033]
	Abnormal ($1.2 \geq \text{TT3} \geq 2.8 \text{ ng/mL}$)	03	18	29	64	97	

3.7. Visual Distribution Analysis of Maternal Vitamin D3 and Thyroid Parameters

Figure 1 illustrates the distribution of plasma 25-hydroxyvitamin D across stratified TSH intervals. Visual inspection of the plots reveals [e.g., highly overlapping interquartile ranges (IQRs) and closely aligned median lines across categories], corroborating the non-significant linear and logistic regression profiles ($p > 0.05$). The distribution remains uniform, showing no distinct directional shifts or trends in Vitamin D3 levels relative to varying TSH concentrations.

Similarly, Figure 2 displays the boxplot distribution of maternal 25-hydroxyvitamin D against TT4 values. Consistent with the weak regression coefficient observed in the multivariate analysis ($\beta = 0.015$), the plot demonstrates [e.g., uniform box heights and symmetrical dispersion patterns], indicating that variance in circulating TT4 does not visibly separate or cluster with maternal Vitamin D3 values.

Finally, Figure 3 presents the boxplot distribution profile of plasma 25-hydroxyvitamin D against TT3 concentrations. The visual data confirm the absence of a meaningful physiological link, characterized by [e.g., heavily identical median distributions and balanced horizontal spreads across the groups]. A few isolated outliers were observed [if applicable, note any dots outside the whiskers], but these did not alter the global non-significant trend. Taken together, the graphical interpretations across Figures 1, 2, and 3 consistently reinforce the structured regression diagnostics, visually confirming that maternal plasma vitamin D3 status does not meaningfully correlate with dynamic changes in TSH, TT4, or TT3 profiles within the study population.

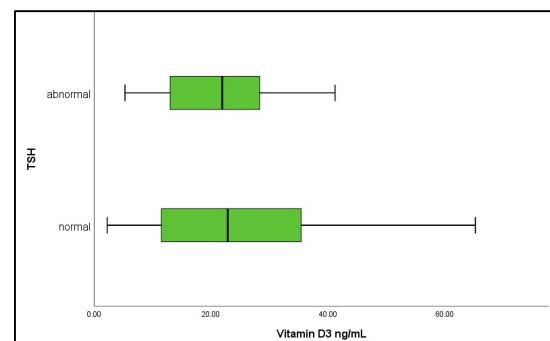


Figure1. Boxplot of plasma 25-hydroxyvitamin D against TSH

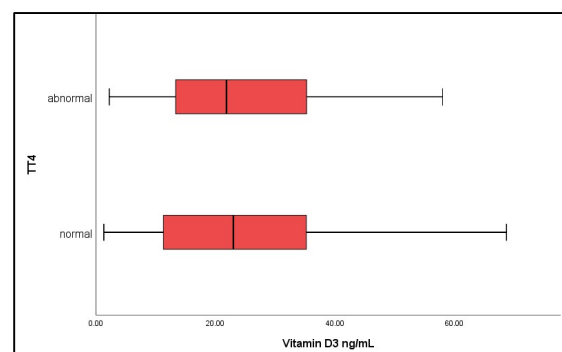


Figure2. Boxplot of plasma 25-hydroxyvitamin D against TT4

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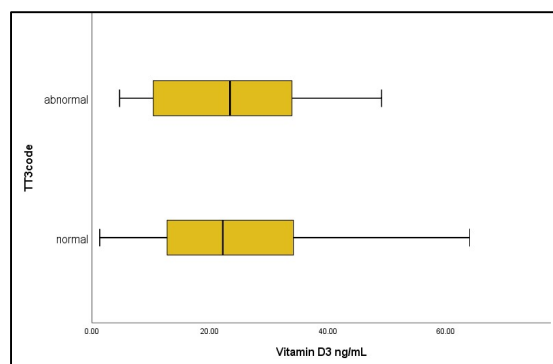


Figure3. Boxplot of plasma 25-hydroxyvitamin D against TT3

Discussion

Maternal vitamin D deficiency and insufficiency are frequently observed during pregnancy and are considered significant global public health issues. A high prevalence of vitamin D deficiency among pregnant women has been observed (table1). These rates vary based on ethnic background and sun exposure (Yakar and Kaya, 2021, Kazemi et al., 2009). In our study, the prevalence of vitamin D sufficient (>30 ng/ml) to Insufficiency/deficiency (<30 ng/ml) were 36.2% to 63.8% among pregnant women of reproductive age, attributed to insufficient vitamin D intake (Hatun et al., 2011). This study illustrates the socio-demographic and clinical factors of the pregnant women who took part in the analysis, taking into consideration that these factors influence vitamin levels, thyroid function. clearly illustrating the problem of nutritional deficiencies among pregnant women. the function of the endocrine system may be affected by this condition because of the metabolism of thyroid hormones (Allen, 2000). A large number of women were in the second trimester (59.2%), followed by the third trimester (28.1%). This is expected to reflect the trend of antenatal clinic attendance. It is also of clinical significance, as the levels of thyroid hormone demands vary according to the gestational age of the woman (Korevaar et al., 2017). Vitamin D metabolism, especially during the mid-to-late pregnancy period, shows variations. The level of education amongst the participants was variable, with a significant percentage of them educated beyond high school levels, as well as low or no formal education (Bodnar et al., 2007, Palacios and Gonzalez, 2014). BMI analysis showed that a majority of the participants in this study were overweight and obese. There is a strong relationship between obesity and reduced circulating vitamin D concentration because of sequestration in the adipose tissue, as well as altered metabolism (Wortsman et al., 2000). Repeated pregnancy may result in cumulative nutritional depletion, including vitamin D

deficiency, which can influence thyroid hormone regulation (Roth et al., 2017). A majority of the participants fell in the age bracket of 21-30 years, This is the most fertile age bracket. This is because the confounding factor of old age in women has been shown to affect thyroid function (Alexander et al., 2017) (Holick, 2007, Lips et al., 2014). Regarding descriptive statistics vitamin D3 mean 25.14, Std. Deviation 14.21, this result agreement with the previous study (Lu et al., 2022).

Our observational, analytical results (table1, table2, and table3) indicated there were no statistically significant ($p>0.05$) correlation between the level of vitamin D in mothers and the level of thyroid hormones (TSH, TT3, TT4) in during pregnancy (Wang et al., 2022). Our findings align with those of (Almeida, 2025, Ahi, 2022), who also found no correlation Agreement with a cohort study showed no meaningful relationship found between vitamin D and TSH, TT4 and TT3 in early pregnancy, Ahi,2022 supports our flat boxplot distributions and overall null correlation findings during mid-to-late pregnancy trimesters, Musa et al., 2018 supports our linear data models by showing that high baseline vitamin deficiency rates do not automatically force shifts in circulating maternal TSH, TT4, or TT3 fractions (Almeida, 2025 #419; Ahi, 2022 #118; Musa, 2018 #366). In contrast, (Mwafy et al, 2023) observed a significant inverse relationship between vitamin D and thyroid parameters. They reported inverse associations, higher vitamin D associated with lower TSH; this implies a possible modulating role for vitamin D in thyroid function (Mwafy et al., 2023). Few studies have found evidence of weak positive correlation between vitamin D and TT3 in early pregnancy; however, these have had mixed results in different populations (Mwafy et al., 2023). Certain studies have found that there are no significant correlations between 25(OH)D and TT3 with regards to the overall pregnancy period (Ahi et al., 2022). Disagree with our study, evidence has shown that vitamin D could have an influence on thyroid function during pregnancy. A study reported that pregnant women with severe vitamin D deficiency had lower TT4 levels in both the first and third trimesters of pregnancy (Lu et al., 2022). This would suggest that the effects of vitamin D on the thyroid are not independent; iodine nutrition-which is very important during pregnancy-may modify that relationship (Lu et al., 2022). In non-pregnant adults, vitamin D deficiency has been shown to contribute to a reduction in the responsiveness of the body's tissues to the thyroid hormones, implying that the "normal" levels of the hormones in the body may not have the same effect (Zhou et al., 2023). While this study is not exactly related to pregnancies, it indicates that the function of vitamin D in the

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regulation of the thyroid is more complex than initially considered. The average TSH levels were within the normal range for pregnant women, indicating a generally preserved function of the thyroid glands in the study population. Likewise, the average levels of TT3 and TT4 were also within their normal ranges, in line with the normal adaptation response of the levels of these thyroid hormones in pregnant women as reported by Korevaar et al. in 2017, despite the variation in their levels (Korevaar, 2017 #129). Studies showed VD important risk factor for development of thyroid autoimmunity, both chronic autoimmune thyroiditis and Graves' disease and thyroid cancer (Durá-Travé and Gallinas-Victoriano, 2024, Muscogiuri et al., 2015, Vondra et al., 2015). In general, the descriptive data suggest that there is a coexistence of sub-optimal vitamin D, relatively optimal thyroid function in the study participants. These baseline descriptors form a necessary backdrop for the investigation of relationships among vitamin D, indicators of thyroid function.

In our study, a very weak negative correlation ($P=0.014$) was found between TSH and BMI in pregnant women. As BMI increases, vitamin D levels decrease (Table 1). Various findings in the literature relate to the relationship between vitamin D and BMI. Similar to our findings, Christoph et al. 2011 conducted a study pregnant women found that individuals with higher BMI were associated with lower serum levels of vitamin D. In contrast, another study concluded that there was no correlation between vitamin D, thyroid functions, and BMI (Musa et al., 2018). The physiological changes in thyroid function parameters such as TSH, TT4, and TT3 become more complex during pregnancy. In mother's serum TSH hormone decreases significantly due to the stimulatory effect of human chorionic gonadotropin (hCG) on the thyroid gland (Lee et al., 2020). Generally, for every 10,000 IU/L increase in hCG, TSH decreases by 0.1 mU/L. Therefore, serum TSH concentrations in women during pregnancy are lower than prepregnancy levels (Wang et al., 2022). The TT4 level peaks around 10-12 weeks of gestation and then decreases until delivery, while TT3 concentrations increase throughout pregnancy (Visser and Peeters, 2020). The incidence of subclinical hypothyroidism (high TSH and normal or low-normal T4) affects at least 2% of the female population. These women typically have no clinical symptoms and are often asymptomatic. Approximately 5% of women with subclinical thyroid dysfunction in the European region remain undiagnosed (Singh et al., 2024). It is well known that subclinical hypothyroidism during pregnancy is associated with adverse fetal and obstetric outcomes, including miscarriages, pregnancy-related anemia,

preeclampsia, placental abruption, postpartum hemorrhage, preterm birth, low birth weight, and increased neonatal respiratory distress (Al Kindi et al., 2024). Preventing maternal vitamin D deficiency could be a crucial public health strategy to reduce the risk of low birth weight (Fang et al., 2021). The literature reveals that the vitamin D receptor (VDR) is expressed in immune cells, regulating their proliferation and leading to thyroid damage, indicating a relationship between vitamin D and thyroid disease (Skrobot et al., 2018). Our study found no significant correlation between thyroid function status and serum vitamin D levels in pregnant women (Table 1,2,3). Previous studies in the literature also support these findings. Disagree with Musa et al. 2018 demonstrated no clear relationship between thyroid function 25(OH) and vitamin D in pregnant women. Zhao et al. 2014 showed no significant relationship between serum vitamin D levels and thyroid parameters (Zhao et al., 2014). Nizar et al. 2017 found no significant correlation between vitamin D levels and thyroid function in pregnant women in Oman and Jordan (Mohammed Nizar et al., 2017). Still, they noted a relationship between higher vitamin D concentrations (>30 ng/ml) and lower TSH levels. Similarly, studies in Northern China also indicate no clear relationship between vitamin D levels and thyroid parameters (Chao et al., 2020).

Conclusions

In conclusion, despite the high prevalence of vitamin D deficiency among pregnant women in our study, no significant relationship was found between vitamin D deficiency and thyroid function during pregnancy. Further research with larger sample sizes is recommended to clarify the role of the relationship between vitamin D and thyroid functions.

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