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The Impact of First-Trimester Vitamin D Levels On Gestational Diabetes Mellitus

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ABSTRACT

This study aims to investigate the association between first-trimester vitamin D levels and the development of gestational diabetes mellitus (GDM) in a Turkish population.

A retrospective cohort study was conducted involving 458 pregnant women who attended antenatal screening at the University Medical Faculty Department of Obstetrics and Gynecology between March 2018 and December 2019. Vitamin D levels were measured during the first trimester, and GDM was diagnosed using a two-step oral glucose tolerance test (OGTT) approach between 24-28 weeks of gestation. Statistical analyses, including binary logistic regression, were performed to compare vitamin D levels and other variables between women with and without GDM.

Of the 458 participants, 9.8% were diagnosed with GDM. The mean age and body mass index (BMI) were significantly higher in the GDM group compared to the non-GDM group ($p<0.001$). Despite a high prevalence of vitamin D deficiency and insufficiency (86% and 97.4%, respectively), no significant difference was found in the vitamin D levels between women with GDM and those without ($p>0.05$). In the multivariate logistic regression analysis, age (OR: 1.076, $p=0.040$) and BMI (OR: 1.094, $p=0.006$) were identified as independent risk factors for GDM, whereas first-trimester vitamin D levels were not associated with GDM risk ($p=0.617$).

The study did not find a significant relationship between first-trimester vitamin D levels and the development of GDM after adjusting for confounding factors. The high prevalence of vitamin D deficiency in the population, along with universal vitamin D supplementation, may have influenced these findings. Further prospective studies with larger sample sizes are needed to clarify the potential role of vitamin D in GDM development in different populations.

Keywords: Gestational Diabetes Mellitus (GDM), Vitamin D Deficiency, First Trimester, Pregnancy, Insulin Resistance

Introduction

Gestational diabetes mellitus (GDM) is a widespread condition among pregnant women; its prevalence increases with maternal age, unhealthy lifestyle, and body mass index. In women with GDM, steroid hormone levels increase, insulin resistance occurs in peripheral and adipose tissue, and the placenta secretes inflammatory cytokines. The Hyperglycemia and Adverse Pregnancy Outcome study (HAPO) pointed at a strong relationship between maternal glucose levels and cesarean delivery, neonatal hypoglycemia, and fetal hyperinsulinemia (1). Pregnancies complicated by GDM have a higher risk of developing preeclampsia (2); furthermore, there is an increased risk of developing diabetes in the future (3). The fetus is also at increased risk for macrosomia, neonatal hypoglycemia, hyperbilirubinemia, shoulder dystocia, and birth trauma (4).

The human body obtains vitamin D through diet and sun exposure. Vitamin D is involved in bone metabolism and calcium management. Various studies showed that it also has extra-musculoskeletal impacts such as on the immune system, cell proliferation, insulin resistance, and beta-cell function (5, 6). A recent meta-analysis of observational studies estimated that low vitamin D status is associated with an increased risk of GDM (7).

It is also known that vitamin D deficiency is a frequent public health issue. Several countries suffer from vitamin D deficiency in pregnancy (8-10). Despite a sunny environment, maternal vitamin D deficiency is still a fundamental health problem in Türkiye (10). This study aims to evaluate the possible link between vitamin D levels and GDM in the Turkish population.

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Materials and Methods

This retrospective cohort study analyzed data from pregnant women who were admitted to the University Medical Faculty, Department of Obstetrics and Gynecology between March 2018 and December 2019 for antenatal screening in their first trimester. The study was approved by the Institutional Review Board (IRB approval I5-282-20, date 08.06.2020).

During the study period, pregnant women were assessed for eligibility by reviewing hospital records. Socio-demographic information including age, obstetric history, body mass index, diabetes mellitus, and chronic illnesses was obtained from patient records. Gestational age was determined based on the last menstrual cycle date and confirmed through ultrasonographic measurements (Voluson E8, GE Healthcare).

Women with multiple fetuses (e.g., twins or triplets), clinically diagnosed chronic illnesses (diabetes mellitus, hypertension, cardiac disease or thyroid disease), positive test results for hepatitis B surface antigen, HIV or syphilis, and those taking medications or undergoing assisted pregnancy were excluded from the study.

All pregnant patients undergoing antenatal screening had their weight and height measured. We used the HPLC method with a Thermo Scientific Dinex 3000 RS Liquid Chromatography to measure plasma baseline 25-hydroxyvitamin D levels during the antenatal test. Biochemical analyses were done using a Beckmann Coulter 5800 autoanalyzer system, while hormone levels were measured with a Beckmann Unicel DXI 800 immunoassay. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m2). After the first trimester, all patients received regular supplemental vitamin D at a dose of 1000-1200 IU (9 drops) per day.

The OGTT, which screens for gestational diabetes, was performed between weeks 24 and 28 of pregnancy. All laboratory studies were conducted on the same day. In this study, mothers with vitamin D levels below 12 ng/mL were classified as having severe deficiency, while those with levels below 20 ng/mL were considered deficient. Insufficiency was defined as levels below 30 ng/mL.

A total of 458 pregnant women at 24–28 weeks of gestation underwent a screening test known as the 50-g OGTT. The test is typically conducted between weeks 24 and 28 in a non-fasting state. If the screening threshold was met or exceeded (140 mg/dL [7.77 mmol/L]), patients underwent the 100-g oral glucose tolerance test. During the OGTT, a fasting glucose level is obtained followed by the consumption of a 100-g glucose load; then their blood sugar levels are evaluated post-consumption for up to three hours. A diagnosis of GDM was confirmed using the Carpenter-Coustan criteria when two or more glucose values fell at or above specified thresholds (4, 11).

Statistical Analysis: Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 11.5 (SPSS Inc., Chicago, IL, USA). Continuous variables were assessed for normality using the Kolmogorov-Smirnov test and normal probability plots. Descriptive statistics were presented as mean ± standard deviation (SD) for normally distributed variables, median (minimum-maximum) for non-normally distributed variables, and number (percentage) for categorical variables. Comparisons between the GDM and non-GDM groups were conducted using the independent samples t-test for normally distributed continuous variables and the Mann-Whitney U test for non-normally distributed data. Categorical variables were compared using the Chi-Square test. To identify independent risk factors for GDM, a binary logistic regression analysis was performed. The model included GDM as the dependent variable, while age, BMI, family history of type 2 diabetes, parity, and first-trimester vitamin D levels were entered as independent variables. An *a priori* power analysis was not conducted to determine the sample size; however, all eligible patients within the study period were included to maximize statistical power. The lack of a prior power analysis is acknowledged as a limitation of the study. A p-value <0.05 was considered statistically significant for all analyses.

Results

For first-trimester screening, 1.191 pregnant women were admitted to the University Medical Faculty, Department of Obstetrics and Gynecology between December 2018 – March 2019. Data for 220 patients were missing or they had irregular follow-up. 271 patients were excluded due to multiple pregnancy or chronic diseases. Sixty-eight women had pregnancy loss. 174 women did not have any screening test for GDM. Finally, 458 eligible women were enrolled (Figure 1).

The baseline characteristics at recruitment for the women diagnosed with GDM and those with average plasma glucose are shown in Table 1. The mean age of patients with and without gestational diabetes was significantly different ($p<0.001$). Women with GDM were significantly older (30.4 vs. 27.5 years). A difference was found between the BMI averages of patients with and without gestational diabetes

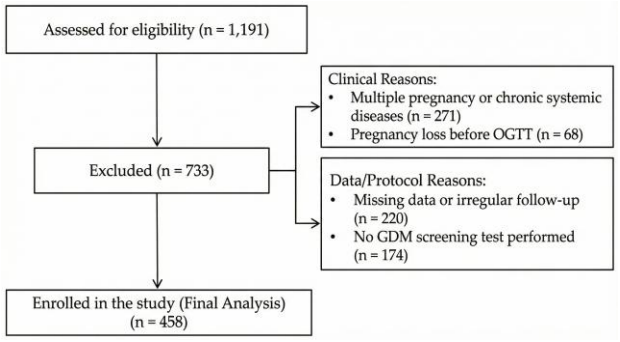


Fig. 1. Flow diagram of the study participants. The flowchart illustrates the recruitment and selection process of pregnant women attending first-trimester antenatal screening. Out of 1.191 women initially assessed for eligibility, participants were excluded based on clinical criteria (e.g., multiple pregnancies, chronic diseases) or data irregularities (e.g., missing follow-up, lack of screening), resulting in a final sample size of 458 included in the analysis. **Abbreviations: GDM:** Gestational Diabetes Mellitus; **OGTT:** Oral Glucose Tolerance Test.

($p<0.001$). The average BMI of patients with GDM was significantly higher than patients without GDM.

When BMI levels were categorized, a difference was found between BMI levels of patients with and without GDM ($p<0.05$). In patients with gestational diabetes, the rate of BMI over 30 was higher than those without gestational diabetes. When the patients were categorized as nulliparous and multiparous, a difference was found between the patients with gestational diabetes and those without ($p<0.05$). Parity was significantly higher in patients with gestational diabetes. Between patients with and without gestational diabetes, the first-degree family history of type 2 diabetes was different ($p<0.05$). The rate of having type 2 diabetes in the family was significantly higher in patients with gestational diabetes.

There was a high proportion of vitamin D deficiency and insufficiency. Overall, 97.4% of mothers were vitamin D insufficient, 86% deficient, and 60.7% were severe vitamin D deficient. No difference was found between the vitamin D levels of patients with gestational diabetes and those without ($p>0.05$).

Binary logistic regression analysis was performed to determine independent risk factors for GDM (Table 3). The model included age, BMI, family history of diabetes, parity, and first-trimester Vitamin D levels. While advanced maternal age (OR: 1.076, 95% CI: 1.003–1.154, p=0.040) and higher BMI (OR: 1.094, 95% CI: 1.026–1.167, p=0.006) were identified as significant independent predictors of GDM, no significant association was found between first-trimester Vitamin D levels and GDM development (OR: 0.988, 95% CI: 0.945–1.034, p=0.617) after adjusting for confounders.

The study participants were divided into groups based on the cut-offs of vitamin D levels (Table 2). Vitamin D levels were 12 ng/mL and below in 68.9% of patients with gestational diabetes, and vitamin D levels were 12 and below in 59.8% of patients without gestational diabetes. No significant difference was found between those with and without gestational diabetes in patients with vitamin D levels below 12 ($p>0.05$). There was no significant difference between gestational diabetes mellitus in patients with vitamin D levels below 20 ($p>0.05$). A similar analysis was performed using the vitamin D cut-off of 30 to categorize the women as insufficiency groups. There was no significant difference in the percentage of GDM in women with vitamin D insufficiency (<30) and women without vitamin D insufficiency (≥ 30) ($p>0.05$).

Discussion

In this study, we interrogated the link between gestational diabetes mellitus and first-trimester vitamin D deficiency. The potential association between vitamin D deficiency (hypovitaminosis D) and insulin resistance may be explained by molecular mechanisms involving the expression of insulin receptors, the production of inflammatory cytokines, and polymorphism of the vitamin D receptor (VDR) in pancreatic β-cells. Specifically, vitamin D regulates gene transcription through both genomic and non-genomic pathways. Based on these mechanisms, vitamin D deficiency and insulin resistance appear to be genetically interrelated (12).

We could not find a significant relationship between vitamin D deficiency measured in the first trimester and the development of gestational diabetes mellitus. Importantly, our multivariate logistic regression analysis confirmed that this lack of association persists even after adjusting for potential confounders such as age and BMI.

This may be partly due to the entire population taking routine vitamin D supplements starting from the first trimester. In a study conducted by Bao et al. with 10 years of follow-up and 21,356 patients, pre-pregnancy supplemental vitamin D intake was found to be significantly and inversely associated with the risk of GDM (13). Similar results were observed in patients receiving vitamin D supplementation from the first to second trimester and in patients with 25(OH)D concentrations >12 ng/mL (14). In a randomized controlled trial, patients with at least one risk factor for GDM were prescribed vitamin D and their risk of developing GDM during pregnancy was compared. A

Table 1: Demographic and Clinical Characteristics of The Study Participants Stratified By Gestational Diabetes Status

Parameters	All data (n = 458)	Women with gestational diabetes mellitus (n =45)	Women with normal glucose levels (n=413)	P-value
Age (years)±	27.8±5.1	30.4±5.2	27.5±5.0	<0.001
BMI (kg/m2) ±	25.2±4.7	27.7±5.0	24.9±4.5	<0.001
BMI categories, N (%)				
<18.5	21 (4.5)	0 (0)	21 (5.1)	0.023
18.5–24.9	222 (48.5)	17 (37.8)	205 (49.6)	
25.0–29.9	145 (31.7)	15 (33.3)	130 (31.5)	
>30	70 (15.3)	13 (28.9)	57 (13.8)	
Nulliparous	173 (37.8)	9 (20)	164 (39.7)	0.010
Multiparous	285 (62.2)	36 (80)	249 (60.3)	
First degree family history of type 2 diabetes, N (%)				
No	257 (56.1)	18 (40)	239 (57.9)	0.022
Yes	201 (43.9)	27 (60)	174 (42.1)	

Note: Continuous variables were compared using the independent samples t-test or Mann-Whitney U test. Categorical variables were compared using the Chi-Square test. Data are presented as mean ± standard deviation (SD), number (percentage), or median (minimum-maximum). **BMI:** Body Mass Index; **GDM:** Gestational Diabetes Mellitus. p<0.05 indicates statistical significance

Table 2: Comparison of First-Trimester 25-Hydroxyvitamin D Levels And Deficiency Prevalence Between Women With and Without Gestational Diabetes

Parameters	All data (n = 458)	Women with gestational diabetes mellitus (n =45)	Women with normal glucose levels (n=413)	P-value
Vit D levels Median (Min-Max)	10.5 (5 – 53.05)	10.7 (5- 53.1)	8.7 (5.0 – 31.7)	0.240
Vit D categories				
Vit D ≤12 ng/mL	278 (60.7)	31 (68.9)	247 (59.8)	0.236
Vit D >12 ng/mL	180 (39.3)	14 (31.1)	166 (40.2)	
Vit D categories				
Vit D ≤20 ng/mL	394 (86)	36 (80)	358 (86.7)	0.691
Vit D >20 ng/mL	64 (14)	9 (20)	55 (13.3)	
Vit D categories				
Vit D ≤30 ng/mL	446 (97.4)	44 (97.8)	302 (96.5)	1.000
Vit D >30 ng/mL	12 (2.6)	1 (2.2)	11 (3.5)	

Note: Continuous variables were compared using the Mann-Whitney U test. Categorical variables were compared using the Chi-Square test. Data are presented as median (minimum-maximum) or number (percentage). **GDM:** Gestational Diabetes Mellitus. Vitamin D deficiency was defined as <20 ng/mL and insufficiency as <30 ng/mL. *p<0.05 indicates statistical significance*

Table 3: Multivariate Binary Logistic Regression Analysis İdentifying İndependent Risk Factors Associated With Gestational Diabetes Mellitus

Variable	Odds Ratio (OR)	95% Confidence Interval	P-value
Vitamin D	0.988	0.945 – 1.034	0.617
Age	1.076	1.003 – 1.154	0.040*
BMI	1.094	1.026 – 1.167	0.006*
Family History of DM	1.820	0.953 – 3.477	0.070
Parity (Multiparity)	1.495	0.639 – 3.501	0.354

Note: Multivariate binary logistic regression analysis was performed. The model was adjusted for maternal age, BMI, family history of type 2 diabetes, parity, and vitamin D levels. **OR:** Odds Ratio; **CI:** Confidence Interval; **BMI:** Body Mass Index. Bold values indicate statistical significance ($p<0.05$)

decrease in the risk of GDM was observed in the intervention group when the group receiving 5000 units of vitamin D daily was compared with the placebo group (15).

In our study, 9.8% of the patients were diagnosed with GDM during their pregnancy. However, in a meta-analysis conducted in our region, the prevalence of GDM in Central Anatolia was found to be 5.1% (16). Similarly, in a recent study examining the relationship between GDM and first-trimester vitamin D deficiency, the GDM rate was determined to be 5.7% (17). The fact that we have higher GDM rates than in our region may be due to the fact that the study was conducted in a reference center and included a high number of patients with high BMI.

Also, we found that 86% of the patients in this study had vitamin D deficiency (16). The group's vitamin D median was determined as 10.7 ng/mL, which is much lower than what was previously determined by Özdemir et al.(18). This may be due to the study being carried out in the winter and spring periods. The ubiquitously low vitamin D levels in this seasonal cohort might have created a 'floor effect', limiting the statistical power to detect a difference between groups.

Our findings may be considered to be in conflict with three separate meta-analyses in the literature (Zhang, Aghajafari, Wei) (19-21). Zhang et al. reported that vitamin D deficiency increased the risk of GDM by 2.66 times. Lacroix et al. found a significant correlation between first-trimester vitamin D levels and GDM in their study (22). However, the possible effect of universal vitamin D supplementation given early in our study should not be ignored. In addition, Makgoba et al. also found no significant association between first-trimester vitamin D levels and GDM in their prospective study (23). Equivalent results were obtained in the studies of Farrant et al. and Park et al. (24, 25). However, it is unclear whether the patients in these studies received vitamin D supplements.

There may be several arguments for this difference. First of all, the vitamin D levels of the studied population were generally deficient. Baker et al. could not find a relationship between first-trimester vitamin D levels and GDM in their study, and they attributed this to higher than expected vitamin D levels (26). Likewise, our low values may have caused us to find no significant difference. Limit values for vitamin D levels may have led to this conclusion. Each society determining its limit value will cause us to define vitamin D deficiency more accurately. It is also crucial to acknowledge that current reference ranges for vitamin D are primarily established based on bone health outcomes. The optimal threshold for extra-skeletal effects, such as glucose homeostasis and insulin sensitivity, remains undefined. This situation creates a paradox, particularly in sunny Mediterranean regions where "deficiency" based on skeletal standards is reported at rates as high as 75-80%, yet does not always correlate with adverse metabolic outcomes. Consequently, the lack of association found in our study might be due to the fact that the cut-off values used for bone health are not applicable to the pathophysiology of GDM. It is possible that the vitamin D levels required for metabolic functions differ significantly from those required for maintaining bone density. Besides, the GDM diagnostic criteria used may affect the outcome of the research. Another point is that all pregnant women started vitamin D replacement in the first trimester, potentially reducing the effect of initial deficiency.

The low number of participants in the study, being in the same region and a single seasonal period, can be considered a limiting factor. Another interesting part of the analysis is that vitamin D deficiency is particularly frequent even though the region is located in a sunny geography. Nevertheless, it should not be forgotten that the examination was made during the

winter period. Lack of plasma insulin and HbA1c levels can be shown as the drawbacks of the study. The retrospective design of the study and the exclusion of patients with incomplete or irregular follow-up constitute the primary limitations. Additionally, an *a priori* power analysis was not performed to calculate the sample size; however, the study included a substantial number of participants from a reference center. Lack of plasma insulin and HbA1c levels can be listed as other drawbacks of the study.

As the strengths of the study, the vitamin D levels of the patients were examined before GDM diagnosis. One of the strengths of our study is that we evaluated vitamin D levels by classifying them as insufficiency and deficiency. Furthermore, we utilized logistic regression to control for confounders, providing more robust evidence regarding the lack of independent association in this specific cohort. Also, it is one of the few studies examining the relationship between vitamin D deficiency and GDM in Türkiye. There is a need for more extensive prospective studies based on the Turkish population and the determination of vitamin D limit levels on a population basis.

This study demonstrated that first-trimester vitamin D levels were not significantly associated with the development of GDM in a Turkish cohort, even after adjusting for independent risk factors such as maternal age and BMI. While advanced maternal age and higher BMI were confirmed as significant predictors of GDM, vitamin D status did not show an independent correlation. The lack of association observed in this study may be attributed to the "floor effect" caused by the high prevalence of vitamin D deficiency in the study population during the winter-spring period, as well as the potential masking effect of universal vitamin D supplementation initiated early in pregnancy.

These findings suggest that in populations with widespread vitamin D deficiency and routine supplementation, first-trimester vitamin D levels may not serve as a reliable early marker for GDM prediction. Future prospective studies with larger sample sizes, covering all seasons and controlling for supplementation dose and duration, are needed to clarify the complex relationship between vitamin D status and glucose metabolism in pregnancy.

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