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RELATIONSHIP BETWEEN GESTATIONAL DIABETES MELLITUS AND VITAMIN D, CALCITONIN AND PARATHORMONE

GESTASYONEL DİYABETES MELLİTUS İLE D VİTAMİNİ, KALSİTONİN VE PARATHORMON İLİŞKİSİ

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ABSTRACT

AIM: There is growing evidence that Vitamin D (vitD) has an important role in glucose metabolism. Although there are some observational studies reporting lower levels of 25 hydroxy vitamin D in pregnant women with gestational diabetes mellitus (GDM) compared to normal pregnant women, the other calcium metabolism hormones calcitonin (Cal) and parathyroid hormone (PTH) have not been fully researched in GDM. The aim of this study was to investigate the effects of vitD, Cal and PTH in GDM.

MATERIAL AND METHODS: A total of 100 pregnant women whose ages and body mass indices were similar, fifty with normal glucose tolerance (NGT) and the other fifty with GDM, were included in the study. Their demographic and anthropometric parameters, vitD, active vitD, Cal and PTH levels in the summer season were screened retrospectively. The pregnant women with GDM were classified according to the age, parity, being veiled, BMI and vitD levels. All parameters were compared, then the correlations of those parameters were investigated in the groups and GDM subgroups.

RESULTS: VitD levels were statistically insignificantly low in the GDM group and also in the older, multiparous, veiled, and obese GDM subgroups. Cal and PTH levels were not different in both groups. The vitD deficient and obese GDM subgroups had significantly higher Cal levels than their opposite subgroups. There was a positive correlation between Cal and BMI in the GDM group and in the VitD deficient, older, and veiled GDM subgroups. Positive correlation between vitD and C-peptide was found only in the obese GDM subgroup. In the logistic regression analysis including GDM subgroups and calcium metabolism hormones, except younger age (OR=0.116 (95% CI=0.04-0.338, p=<0.001)), VitD, active VitD, Cal, and PTH had no effect on GDM prediction.

CONCLUSION: Although no significant difference was found in the VitD, Cal and PTH levels of the GDM patients during the summer season, it was thought that VitD could play a role in the obese GDM patients. It was also concluded that Cal and PTH did not have roles in gestational diabetes mellitus. Larger, carefully designed studies including throughout the pregnancy and postpartum periods and seasonal variations are required.

Key words: *Gestational diabetes, Vitamin D, Calcitonin, Parathyroid hormone*

ÖZET

AMAÇ: D vitamininin (VitD) glukoz metabolizmasında önemli rol oynadığına dair kanıtlar artmaktadır. Gestasyonel diabetes mellituslu (GDM) gebe kadınlarda normal gebe kadınlara kıyasla 25 hidroksi vitamin D düzeylerinin daha düşük olduğunu bildiren bazı gözlemsel çalışmalar olmasına rağmen, diğer kalsiyum metabolizma hormonları Kalsitonin (Kal) ve paratiroid hormonu (PTH) GDM'de tam olarak araştırılmamıştır. Bu çalışmanın amacı GDM'de VitD, Kal ve PTH'nın etkilerini araştırmaktır.

GEREÇ VE YÖNTEMLER: Çalışmaya yaşları, ve vücut kitle indeksleri VKİ) benzer olan ellisi NGT ve diğer ellisi GDM olan toplam 100 gebe kadın dahil edildi. Yaz sezonundaki demografik ve antropometrik parametreleri, vitD, aktif vitD, Kal ve PTH seviyeleri retrospektif olarak tarandı. GDM'li gebeler yaş, parite, örtünme durumu, VKİ ve VitD düzeylerine göre sınıflandırıldı. Tüm parametreler karşılaştırıldı, daha sonra GDM ve GDM alt gruplarında tüm parametrelerin korelasyonları araştırıldı.

BULGULAR: VitD düzeyleri GDM grubunda ve ayrıca ileri yaş, multipar, örtülü ve obez GDM alt gruplarında istatistiksel olarak anlamsız derecede düşüktü. Kal ve PTH düzeyleri her iki grupta farklı değildi. D vitamini eksikliği olan ve obez GDM alt gruplarının Kal seviyeleri, kendi karşıt alt gruplarından belirgin olarak daha yüksekti. GDM grubunda, VitD eksik, yaşlı ve örtülü GDM alt gruplarında Kal ile VKİ arasında pozitif bir korelasyon vardı. VitD ile C-peptid arasında ise sadece obez GDM alt grubunda pozitif korelasyon saptandı. GDM alt grupları ve kalsiyum metabolizma hormonlarını içeren lojistik regresyon analizinde, genç yaş hariç (OR= 0.116 (% 95 CI = 0.04-0.338, p = <0.001)), VitD, aktif VitD, Kal ve PTH'nin GDM'yi öngörme üzerine etkisi yoktu.

SONUÇ: Yaz mevsimindeki GDM hastalarında VitD, Kal ve PTH seviyelerinde anlamlı bir fark bulunamamasına rağmen, VitD'nin obez GDM hastalarında rolü olabileceği düşünüldü. Ayrıca Kal ve PTH'nin gestasyonel diabetes mellitusta rolü olmadığı sonucuna varıldı. Tüm gebelik ve doğum sonrası dönemleri ve mevsimsel farklılıkları içeren geniş çaplı prospektif çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Gestasyonel diyabet, D Vitamini, Kalsitonin, Paratiroid hormon*

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The study approval was granted by the ethical committee of the same center (Approval date and number: 10/02/2019-E-19/35). This study was performed according to the Helsinki Declaration 2008.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first detected during gestation. It is associated with increased feto-maternal morbidity and long-term complications. Advanced maternal age, obesity before and during pregnancy, and previous history of GDM are predisposing factors, but its exact etiology is still unknown (1).

In recent years, evidence about the reciprocal interaction between bone and energy metabolism is growing (2). In the light of this new information, we turned our eyes to other hormones in calcium (Ca) metabolism. There have been many reports that vitamin D (VitD) status is associated with insulin sensitivity, glucose tolerance, and β-cell function in addition to its well-known role in bone metabolism (3,4). Although there are also publications with the opposite idea (5-7), some observational studies reported lower 25 hydroxy vitamin D levels in pregnant women with GDM in comparison to normal pregnant women (8-13). Parathyroid hormone (PTH) is the other main hormone in bone and calcium metabolism. High serum concentrations of PTH are found in diabetics (14). Significantly increased serum concentrations of calcitonin (Cal) levels which is another important hormone in calcium metabolism were observed in diabetics (14). There are contradictions about the pregnancy Cal levels.

There are very few studies about Ca metabolism hormones in GDM. Cal and PTH were not fully investigated in pregnant women with GDM. This study was designed to investigate the role of VitD, Cal and PTH in GDM.

MATERIAL AND METHODS

Patients

Clinical records of 100 pregnant women who were seen in the outpatient clinics of obstetrics and gynecology of Ankara Training and Research Hospital between May 2010 and August 2010 were analyzed retrospectively. Half of the pregnant women had normal glucose tolerance (NGT) and the other half of them had gestational diabetes mellitus (GDM). The study approval was granted by the ethical committee of the same center (Approval date and number: 10/02/2019-E-19/35). This study was performed according to the Helsinki Declaration 2008. The written Informed consent was obtained from all participants.

The patients' demographic features, number of pregnancy and laboratory findings at 24-28th weeks of gestation were obtained from recorded computerized database. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m2). The diagnosis of gestational diabetes was determined by the guidelines proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (15), with a 75 gr 2- hour oral glucose tolerance test at 24-28 weeks of gestation. Pregnant women who had multiple pregnancy, fetal abnormalities, chronic renal, liver or thyroid disease, and consumption of calcium (Ca) supplements or VitD during this pregnancy, and with a history of pregestational diabetes were excluded from the study.

The blood sample was taken after 12 hours of overnight fasting, at 08.30 a.m. for FPG, hemoglobin A1c (HbA1c), creatinine (Cr), alanine aminotransferase (ALT), calcium (Ca), phosphorus (P), magnesium (Mg), PTH, thyroid stimulating hormone (TSH), free thyroxine (fT4), fasting insulin (FI), C-peptide (Cpep), serum total cholesterol (TC), HDL cholesterol (HDL-C), triglyceride (TG), 25 hydroxy vitamin D (25(OH)D), 1,25 dihydroxy vitamin D (1,25(OH)2D), and calcitonin (Cal) levels. The post-prandial blood glucose (PPBG) was determined at the first hour of breakfast. An indirect measure of insulin resistance was calculated from the fasting plasma insulin (μunit/mL) x fasting plasma glucose (mmol/L) /22.5 formula as homeostasis model assessment-insulin resistance (HOMA-IR).

Age, week, and number of pregnancies, and all values of the NGT and GDM groups were documented. Serum 25(OH)D levels less than 20 ng/mL and between 20-29 ng/mL were considered as vitamin D deficiency and insufficiency, respectively (16). After comparing all those parameters, same analyses performed in the GDM subgroups formed according to the age below or above 30 (defined as young or old), BMI below or above 30 kg/m2 (defined as non-obese or obese), 25(OH)D below or above 20 ng/mL (defined as vitD deficient or non-deficient), nulliparous or multiparous and veiled or unveiled. The correlation analyses were also carried out in the GDM group and GDM subgroups mentioned above.

Laboratory Methods

FBG, PPBG, TC, TG, HDL-C, Cr, ALT, Ca, P, Mg were measured by spectrophotometric method in Olympus 2700 autoanalyzer (Mishima Olympus Co. Ltd- Olympus Corporation –Japan) using Olympus System Reagent kits. HbA1c was determined with Tosoh G7 device by HPLC method. LDL-C was calculated by the Friedewald Formula (LDL-C: TC-(TG/5)-HDL-C). TSH, fT4, PTH were studied by chemiluminescence method in Advia Centaur XP (Siemens Diagnostic-USA) device, 25 OHD by Tandem Gold liquid chromatography with mass spectrophotometer (LC/MS/MS- Zivac Technologies- Turkey) device and 1,25(OH)2D with Dvit–Dia Source kits by radioimmune assay method. Cal level was determined by DiaSource kits in DSX automatized ELISA system device (Dynex Technologies Inc USA), FI and Cpep studied by DRG kits in the same device. Corrected Ca was calculated by the formula Corrected Ca: Serum Ca+0.8 (4-Serum albumin). Ca levels mentioned in the text were corrected Ca levels calculated by the formula.

Statistical Analysis

Calculations were performed using SPSS version 11,5

(Customer ID 30000105 930). Continuous variables were expressed as mean ± SD, median (min-max) and categorical variables were expressed as number (n) and percentage (%). The Student's t-test and Mann-Whitney U test were used for the comparison of variables for continuous variables with normal distribution and non-normal distribution, respectively. The Chi square test was applied for in categorical comparisons. The Pearson and Spearman correlation coefficients were used for the correlation analysis of homogeneous and non-homogenous groups, respectively. The logistic regression analysis was employed to assess independent variables that could be associated with development of GDM. A p value < 0.05 was considered significant for all the tests.

RESULTS

Clinical records of 100 pregnant women including 50 NGT and 50 GDM whose ages, body mass indices were similar in 24-28th pregnancy weeks were analyzed. Demographic and laboratory parameters of the groups and their comparisons are shown in **Table 1**. FBG, PPBG, HbA1c, FI, Cpep, HOMA-IR, TG and P levels of the GDM group were significantly higher than the NGT group. In the GDM group, although VitD levels were lower and the percentages of VitD deficiency were higher than the NGT group, there was no significant difference (28.09±12.89 ng/mL vs 31.82±17.12 ng/mL, P=0.482; 44% vs 26%, P=0.059, respectively). PTH, Cal, 1,25(OH)2D, levels and the other parameters were also not statistically different **(Table 1)**. Positive correlations between BMI and Cal (r=0.518, p=0.001) and Cpep (r=0.288, p=0.042) were found in the GDM group **(Table 4).**

Of the GDM patients were 84% multiparous, 76% older, 66% veiled, 44% VitD deficient and 44% obese. The percentages of older and obese subjects in GDM were significantly higher than in the NGT group (p<0.001, p=0.005, respectively). In the VitD deficient GDM subgroup, PTH level was statistically higher and Cal level was significantly lower than the vitD non-deficient GDM subgroup **(Table 3)**. Positive correlations between BMI and Cal (r=0.428, p=0.002) and 1,25(OH)2D (r=0.582, P=0.038), and negative correlation between 25(OH)D and Cal (r=-0.385, p=0.005) were found in this subgroup **(Table 4).**

Table 1. Clinical features and laboratory findings of pregnant women with NGT and GDM

	NGT n:50	GDM n:50	P
Age (year)	29.58±3.98	30.20±3.95	0.437
Week of Pr. (n)	25(24-28)	25(24-28)	0.32
Number of Pr. (n)	2(1-5)	3(1-7)	0.12
BMI (kg/m$^{2)}$	28.19±3.91	29.67±3.69	0.056
FBG (mg/dL)	78.56±6.26	93.90±18.66	**<0.001**
PPBG (mg/dL)	113.34±21.67	160.78±40.97	**<0.001**
HbA1c (%)	5.01±0.29	5.61±0.48	**<0.001**
FI(µu/mL)	11.97±4.34	14.83±5.41	**0.004**
Cpep (ng/mL)	0.86±0.53	1.06±0.54	**0.021**
HOMA-IR	2.35±0.94	3.47±1.55	**<0.001**
25OHD (ng/mL)	31,82±17,12	28,09±12,89	0.482
Vitamin D Deficient (n, %) **Vitamin D Non-Deficient (n, %)**	13, %26 37, %74	22, %44 28, %56	0.059
1,25(OH)$_2$D (ng/mL)	21.70±14.92	22.06±11.74	0.245
PTH (pg/mL)	38.15±21.49	34.37±16.72	0.710
Cal (pg/mL)	9.96±17.16	8.51±8.97	0.408
Ca(mg/dL)	9.45±0.41	9.48±0.48	0.893
Mg(mg/dL)	0.76±0.06	0.75±0.13	0.724
P(mg/dL)	3.26±0.50	3.54±0.47	**0.003**

NGT: Normal glucose tolerant, GDM: Gestational diabetes mellitus, Pr: Pregnancy, BMI: Body mass index, FBG: Fasting blood glucose, PPBG: Post-prandial blood glucose, HbA1c: Hemoglobin A1c, FI: Fasting insulin, Cpep: C-peptide, HOMA-IR: Homeostasis model assessment- insulin resistance index, 25 OHD: 25 hydroxy vitamin D, 1,25(OH$_2$) D: 1,25 dihydroxy vitamin D, PTH: Parathyroid hormone, Cal: Calcitonin, Ca: Corrected calcium, Mg: Magnesium, P: Phosphorus. Data are presented as mean ± SD except week and number of pregnancies which were presented as median (min-max). P is statistical difference between NGT and GDM.

Table 2. Clinical features and glucose metabolism parameters in the GDM subgroups

GDM SUBGROUP		Age (year)	BMI (kg/m²)	Number of Pr. (n)	FBG (mg/dL)	PPBG (mg/dL)	HbA1c (%)	FI (µu/mL)	Cpep (ng/mL)	HOMA-IR
25(OH)D (ng/mL)	**<20 (n=22, 44%)**	29.54 ±4.40	29.37±3.26	**3(1-7)**	94.86±19.87	165.31±50.70	5.65±0.59	13.84±5.19	1.08±0.49	3.19±1.14
	≥20 (n=28, 56%)	30.71±3.55	29.90±4.03	**3(1-5)**	93.14±18.00	157.21±31.89	5.59±0.37	15.61±5.55	1.04±0.59	3.69±1.79
Age (year)	**<30 (n=12, 24%)**	**24.23±2.36*****	**26.90±4.05****	**2(1-3) ***	86.66±14.88	159.66±32.93	5.52±0.71	14.94±4.44	**0.81±0.28***	3.16±0.92
	≥30 (n=38, 76%)	**32.05±2.37*****	**30.54±3.14****	**3(1-7) ***	96.18±19.32	161.13±43.59	5.64±0.39	14.80±5.74	**1.14±0.59***	3.57±1.70
BMI (kg/m²)	**<30 (n=28, 56%)**	**29,21±3,84***	**26,95±2,42*****	**2(1-4) ***	92,17±21,28	165.96±48.21	5.57±0.54	14.89±5.88	**0.89±0.48***	3.44±1.67
	≥30 (n=22, %44)	**31,45±3,81***	**33,12±1,35*****	**3(1-7) ***	96,09±14.89	154.18±29.10	5.67±0.38	14.76±4.88	**1.27±0.56***	3.51±1.42
Parity	**Nulliparous (n=8, 16%)**	30.75±3.80	**26.17±2.79****	**1(1-1) ***	96.00±19.29	167.00±27.50	5.55±0.27	**13.85±5.15****	1.04±0.45	**3.21±1.39****
	Multiparous (n=44, 84%)	30.09±4.01	**30.33±3.48****	**3(1-7) ***	93.50±18.76	159.59±43.22	5.63±0.51	**20.01±3.65****	1.06±0.57	**4.83±1.71****
Veiling	**Unveiled (n=17, 34%)**	31.05±3.39	**27.66±3.71****	**2(1-5) ***	98.94±22.83	180.47±51.88*	**5.87±0.59****	15.06±6.32	1.05±0.56	3.74±1.90
	Veiled (n=33, 66%)	29.75±4.19	**30.70±3.27****	**3(1-7) ***	91.30±15.88	150.63±30.36	**5.48±0.35****	14.72±4.98	1.07±0.55	3.35±1.34

GDM: Gestational diabetes mellitus, BMI: Body mass index, Pr: Pregnancy, FBG: Fasting blood glucose, PPG: Post-prandial blood glucose, HbA1c: Hemoglobin A1c, FI: Fasting insulin, Cpep: C-peptide, HOMA-IR: Homeostasis model assessment- insulin resistance index, 25 OHD: 25 hydroxy vitamin D. Data are presented as mean ± SD except number of pregnancies which were presented as median (min-max). P is statistical difference within each subgroup. ***P<0.05, **P<0.01, *** P<0.001.**

In the obese GDM subgroup, age, number of pregnancies, Cpep and Cal levels were statistically higher than those in the non-obese GDM subgroup **(Table 2 and Table 3).** We found a negative correlation between 25(OH)D and BMI (r=-0.730, P=0.01), and also found positive correlations between 25(OH)D and Cpep (r=0.506, P=0.045), and between BMI and 1,25(OH)2D (r=0.440, P=0.031) in the obese diabetic pregnants **(Table 4).**

In the older GDM subgroup, BMI, number of pregnancies, Cpep levels were significantly higher than the younger GDM subgroup **(Table 2).** The Calcium metabolism hormones were similar in this subgroup **(Table 3).** BMI, FI, HOMA-IR of the multiparous diabetic women were found to be statistically higher **(Table 2)** and VitD levels were significantly lower than those of the nulliparous diabetic women **(Table 3).** The number of pregnancies, BMI, HbA1c, 1,25(OH)2D levels of the veiled GDM patients were statistically higher than the unveiled GDM patients. There was a positive correlation between BMI and Cal in both older and veiled GDM subgroups (r=0.403, p=0.004; r=0.425, P=0.002, respectively) **(Table 4).**

In the logistic regression analysis including GDM subgroups and calcium metabolism hormones, except younger age (OR=0.116 (95% CI=0.04-0.338, p=<0.001)), 25(OH)D, 1,25(OH)2D, Cal, and PTH had no effect on the prediction of GDM.

DISCUSSION

There are many reports that VitD status is associated with insulin sensitivity, glucose tolerance and β-cell function (3,4). However, there are conflicting reports about VitD deficiency and its association with GDM occurrence and related adverse pregnancy outcomes (5-13). In a meta-analysis, Amraei and coworkers found that vitamin D deficiency among mothers may be related to an increased risk of gestational diabetes (OR = 1.18; 95% CI, 1.01–1.35; p <0.001) (17). Similarly, a review on VitD status and GDM risk concluded that maternal VitD deficiency and insufficiency are associated with markers of altered glucose homeostasis (18). In a double-blind randomized controlled clinical trial, which supplemented VitD on pregnant women with GDM, women treated with VitD had a significant increase in VitD serum levels and a significant decrease in fasting glucose, insulin serum and HOMA-IR (19). In contrast to these studies, there are some studies which concluded that there are no significant differences between the VitD status of women with GDM and controls, and VitD deficiency is not a risk factor for GDM (5-7). In the present study, the VitD levels of gestational diabetics were non-statistically lower than normal glucose tolerant pregnant women.

The percentage of VitD deficient patients was 44% in GDM and 26% in NGT, briefly the number of patients with VitD deficiency was non-significantly high in the GDM group.

Table 3. Calcium metabolism parameters in the GDM subgroups

	GDM SUBGROUP	25(OH)D (ng/mL)	1,25(OH)D (ng/mL)	PTH (pg/mL)	Cal (pg/mL)	Ca (mg/dL)	P (mg/dL)
25(OH)D (ng/mL)	**<20 (n=22, 44%)** **≥20 (n=28, 56%)**	**15.68±3.76**** **37.85±8.15****	24.35±14.48 20.27±8.90	**40.09±17.91*** **30.60±14.90***	**5.78±10.67*** **7.38±9.63***	9.56±0.41 9.42±0.52	**3.38±0.66*** **3.66±0.51***
Age (year)	**<30 (n=12, 24%)** **≥30 (n=38, 76%)**	29.42±14.73 27.67±12.44	22.95±10.94 21.78±12.10	28.25±11.64 36.83±17.75	5.91±6.39 9.34±9.57	9.59±0.59 9.45±0.44	3.48±0.42 3.55±0,49
BMI (kg/m²)	**<30 (n=28, 56%)** **≥30 (n=22, %44)**	29.01±2.08 27.37±13.67	19.29±8.55 24.24±13.09	34.48±17.86 35.15±15.55	**5.66±6.503**** **12.14±10.43****	9.56±0.52 9.38±0.41	3.57±0.49 3.44±0.46
Parity	**Nulliparous (n=8, 16%)** **Multiparous (n=44,84%)**	**36.23±10.12*** **26.54±12.87***	23.97±12.80 21.70±11.65	32.61±7.17 35.19±18.00	5.94±5.05 9.09±9.5	9.79±0.63 9.43±0.43	**3.83±0.23*** **3.49±0.49***
Veiling	**Unveiled (n=17, 34%)** **Veiled (n=33, 66%)**	29.97±11.05 27.13±13.81	**27.70±14.30*** **19.16±9.12***	30.50±10.58 39.97±18.90	7.22±7.85 9.18±9.54	**9.79±0.54**** **9.32±0.36****	3.6±0.42 3.47±0.49

GDM: Gestational diabetes mellitus, BMI: Body mass index, 25(OH)D: 25 hydroxy vitamin D, $1,25(OH)_2D$: 1,25 dihydroxy vitamin D, PTH: Parathyroid hormone, Cal: Calcitonin, Ca: Corrected calcium, P: Phosphorus. Data are presented as mean ± SD. P is statistical difference within each subgroup. ***P<0.05, ** P<0.01.**

Table 4. Calcium metabolism parameters in the GDM subgroups

GROUP / SUBGROUP		SIGNIFICANT CORRELATIONS		
	GDM	**BMI-Cal** r=0.518, p=0.001	**BMI-Cpep** r=0.288, p=0.042	
GDM SUBGROUP	**25(OH)D<20 ng/mL**	**BMI-Cal** r=0.458, p=0.002	**BMI-$1,25(OH)_2D$** r=0.582, p=0.038	**25(OH)D -Cal** r= -0.385, p=0.005
	BMI≥30 kg/ m²	**BMI-$1,25(OH)_2D$** r=0.440, p=0.031	**BMI-25(OH)D** r= -0.730, p=0.01	**25(OH)D -Cpep** r=0.506, p=0.045
	Age≥30 year	**BMI-Cal** r=0.403, p=0.004		
	Veiled	**BMI-Cal** r=0.425, p=0.002		

GDM: Gestational diabetes mellitus, BMI: Body mass index, Cal: Calcitonin, Cpep: C peptide, 25(OH)D: 25 hydroxy vitamin D, $1,25(OH)_2D$: 1,25 dihydroxy vitamin D.

The current outcome may have been caused by the season in which the study was made in summer that was the sunniest months of the year (20). In addition, the patients have not received Ca supplements or Vitamin D but have been taking advice during their pregnancy such as daily living conditions, nutrition, simple hygiene conditions, exercise, or maximum utilization of sunlight.

Obesity is an important risk factor for GDM (1), and BMI is a significant predictor of 25(OH)D (21). In the current study, the obese GDM subgroup has lower VitD than the non-obese group but there was no statistical difference. Negative correlation was found between 25(OH)D and BMI in the obese GDM subgroup who had increased number of pregnancies. There is evidence that VitD may stimulate pancreatic insulin secretion directly through its receptors; and the stimulatory effects of vitamin D on insulin secretion may only manifest when calcium levels are adequate (22,23). In this study, concerning VitD and insulin secretion, a positive correlation was found between 25OHD and Cpep levels only in the obese GDM subgroup. These results suggested that vitD may play a role in obese gestational diabetic patients.

It was determined that 1,25(OH)2D levels double early in pregnancy and maintain this increase until term (24). The renal 1-α hydroxylase activity has been shown to be up regulated in the mother. The classic mechanism known is that calcitriol can regulate intracellular calcium flux on β-pancreatic cells and therefore can

modulate depolarization-stimulated insulin release (25). In studies with diabetic mice, high doses of 1,25(OH)2D have been shown to delay the onset of diabetes by protecting β-cell function (26,27). In this study, 1,25(OH)2D levels of the VitD deficient and obese GDM groups were non-statistically high; and there was a positive correlation between 1,25 (OH)2D and BMI. It has been thought that in the obese patients renal 1-α hydroxylase activity was increased for compensation (28). This result may reflect an adaptive mechanism to maximize calcium absorption in these patients. The reason for not finding a significant result or correlation between BMI and 1,25 (OH)2D in the other groups may be due to short half life of 1,25(OH)2D and failure to measure tissue levels that are more important than the serum levels for its action.

Cal is a hypocalcemic and hypophosphatemic hormone decreasing bone resorption by bone cells and calcium reabsorption by the kidneys. Cal levels in human pregnancy have generally been reported to be higher than nonpregnant values (29,30), except some studies (31,32). Higher estradiol, estrone, and estriol levels during pregnancy may stimulate calcitonin synthesis through C cell hyperplasia (25). In animal study, it is suggested that gestation might result in augmented sensitivity to endogenous Cal (33). During pregnancy, women have a positive calcium balance, but considerably less so than the amount estimated to be necessary for the fetus. The increased levels of Cal during gestation suggest that this hormone plays a role in the protection of the maternal skeleton (25). Significantly increased serum concentrations of Cal were found in diabetics (34). In the current study, although Cal levels in the GDM patients was non-significantly high, it was found significantly lower in the Vit D deficient GDM and higher in the obese GDM patients than their own opposing subgroups. Cal and BMI were positively correlated in the GDM patients, in Vit D deficient, older, and veiled GDM subgroups. It was thought that Cal increased to protect the skeleton under conditions of raised Ca demand during pregnancy.

Maternal total calcium levels decrease during pregnancy, with a nadir at 28 to 32 weeks related to the decrease in albumin levels due to increased vascular volume (25). Although some studies have suggested that PTH levels increase during pregnancy, measurements of intact PTH levels by two-site immunometric assay indicate that they are within the normal nonpregnancy range throughout pregnancy (25). On the other hand, PTH-related peptide (PTH-rP), which is synthesized by fetal parathyroid glands and placenta, increases throughout pregnancy (35). PTH-rP could reach the maternal circulation and, after binding to PTH type 1 receptor (PTHR1), it may induce gene expression of CYP27B1 in the kidney and catalyze calcitriol synthesis for endocrine actions (36). Increased serum concentrations of PTH were found in diabetics (14). Nachakar et al found that increased PTH was associated with decreased insulin sensitivity, β-cell function and GDM during pregnancy, regardless of the underlying 25OHD level (37). In the present study PTH levels were not different in all groups. It was thought that the measurement of PTH in combination with placental PTH-rP are necessary for the accurate assessment of glucose metabolism in GDM patients.

This study had some limitations. First, is the moderate sample size in all groups. Second, values evaluated in this study represents only one point in time. Long-term measurements of the hormones can better demonstrate its value in GDM. Third, the study was performed during the summer season, it is obvious that seasonal variations could have influenced the results. Fourth, the gold standard for the measurement of insulin sensitivity is the use of the euglycemic clamp; the insulin resistance was demonstrated by an indirect method, HOMA-IR. Finally, it was not possible to measure the tissue hormone levels, particularly active vitD, for more accurate results to be found.

In conclusion, although no significant difference of Vit D, PTH and Cal levels in GDM patients during the summer season were found in this study, VitD may play a role in obese GDM patients. It was thought that calcitonin and parathyroid hormone did not have roles in gestational diabetes mellitus. Larger carefully designed studies including throughout pregnancy and postpartum and seasonal variations are required.

Conflicts of Interest

The authors declare no conflicts of interest. There is no financial or non-financial interest that may be interpreted to have influenced the manuscript.

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The study approval was granted by the ethical committee of the same center (Approval date and number: 10/02/2019-E-19/35). This study was performed according to the Helsinki Declaration 2008.