Turkish Journal of Diabetes and Obesity / *Türkiye Diyabet ve Obezite Dergisi*
Original Article / Özgün Araştırma

Exploring Serum Vitamin D Binding Protein Levels in Type 1 Diabetes: Assessing the Impact of Glycemic Control and Disease Duration

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Cite this article as: Özcan EG and Doğan D. Exploring serum vitamin d binding protein levels in type 1 diabetes: assessing the impact of glycemic control and disease duration. Turk J Diab Obes 2023;3: 256-262.

ABSTRACT

Aim: Patients with type 1 diabetes mellitus (T1DM) are known to be more prone to vitamin D deficiency. Vitamin D studies in this patient population have traditionally been performed using serum 25OHD levels. However, vitamin D binding protein (VDBP) has been less studied. This study aims to compare serum VDBP levels in T1DM with healthy controls. It also aims to investigate the factors affecting VDBP levels such as disease duration, HbA1c, insulin dose, and age in diabetic subjects.

Material and Methods: A research study was conducted at Çanakkale Onsekiz Mart University Health Practice and Research Hospital. The study included 11-17 years old children with T1DM and healthy controls. Serum VDBP and 25OHD concentrations were compared with appropriate statistical methods according to the normal distribution of relevant parameters. For the diabetic subjects, insulin doses and duration of diabetes were recorded. Spearman's correlation test was utilized to assess associations between continuous variables, and regression analysis was employed to determine predictors of serum VDBP levels.

Results: The study enrolled 89 subjects, including 40 with diabetes. Serum 25OHD levels were similar in the T1DM group and control group (17.03 IQR:12.89-22.08) and (17.62 IQR:11.68-24.48), respectively (p=0.701). However, VDBP levels were significantly lower in the T1DM group (335 µg/ml, IQR: 199.8-517.2 µg/ml) compared to the control group (471.2 µg/ml, IQR: 368.3-533.2 µg/ml) ($p < 0.015$). In the entire group, only the presence of diabetes affected VDBP levels (B=87.236, SE=34.802, p=0.014). On the other hand, HbA1c, duration of diabetes, and insulin dose had no influence on VDBP in the diabetes group.

Conclusion: Serum VDBP levels were significantly lower in T1DM patients but in this group, disease duration, insulin dose, and metabolic control did not affect serum VDBP levels. Serum VDBP concentrations in T1DM may be affected by other parameters rather than metabolic parameters. Therefore, future studies should focus on addressing this knowledge gap.

Keywords: *Vitamin D-binding protein, Vitamin-D, Type 1 diabetes mellitus, Adolescent*

Tip 1 Diyabette Serum Vitamin D Bağlayan Protein Düzeylerinin Araştırılması: Glisemik Kontrol ve Hastalık Süresinin Etkisi

ÖZ

Amaç: D vitamini eksikliği, Tip 1 diyabet mellitus (T1DM) hastalarında yaygın olarak görülür. Tip 1 DM ile D vitamini arasındaki ilişki geleneksel olarak serum 25-hidroksivitamin D (25OHD) seviyeleri aracılığıyla araştırılmıştır, ancak D Vitamini bağlayıcı proteinin (VDBP) rolü daha az incelenmiştir. Bu çalışmanın amacı T1DM'li hastalarda serum VDBP düzeylerini diyabetik olmayan kişilerle karşılaştırmak ve hastalık süresi, HbA1c, insülin dozu ve yaş gibi bu düzeyleri etkileyen faktörleri araştırmaktır.

Gereç ve Yöntemler: Bu çalışma Çanakkale Onsekiz Mart Üniversitesi Sağlık Uygulama ve Araştırma Hastanesi'nde gerçekleştirildi. Çalışma popülasyonu 11-17 yaş arası T1DM çocukları ve sağlıklı kontrolleri içermektedir. Serum VDBP ve 25OHD konsantrasyonları, ilgili parametreler normal dağılım durumuna göre uygun istatistiksel yöntemlerle karşılaştırıldı. Diyabetli olgular için, kullandığı insülin

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DOI: 10.25048/tudod.1402013

Received / *Geliş tarihi* : 08.12.2023
Revision / *Revizyon tarihi* : 26.12.2023
Accepted / *Kabul tarihi* : 26.12.2023

dozları ve diyabet süreleri öğrenildi. Sürekli değişkenler arasındaki ilişkiler Spearman korelasyon testi ile, serum VDBP düzeylerinin öngörücüleri ise regresyon analizi ile değerlendirilmiştir.

Bulgular: Çalışmaya 40 T1DM hastası ve toplam 89 katılımcı dahil edildi. Serum 25OHD düzeyleri T1DM grubunda (17,03 IQR:12,89-22,08) ve kontrol grubunda (17,62 IQR:11,68-24,48) benzerdi (p=0,701). Ancak VDBP düzeyleri T1DM grubunda (335 µg/ml, IQR: 199,8-517,2 µg/ml) kontrol grubuna (471,2 µg/ml, IQR: 368,3-533,2 µg/ml) kıyasla anlamlı derecede düşüktü (p <0,015). Tüm olguları içeren kohortta yalnızca diyabetli olmak VDBP düzeylerinin tek anlamlı belirleyicisidir (B=87.236, SE=34.802, p = 0.014). Öte yandan diyabet grubu içinde HbA1c, diyabet süresi ve insülin dozu VDBP üzerine etkili birer faktör değildi.

Sonuç: Çalışma, T1DM hastaları ile kontroller arasında serum 25OHD düzeylerinde anlamlı bir fark olmadığını göstermiştir. Ancak VDBP düzeylerinin T1DM kohortunda hastalık süresi, insülin dozu ve metabolik kontrolden bağımsız belirgin düşük olduğu görüldü. İncelediğimiz parametreler dışında başka faktörlerin araştırılacağı daha geniş çalışmalar, T1DM'de VDBP değişimi ve bunun genel sağlık üzerine etkilerini daha ayrıntılı ortaya çıkarabilir.

Anahtar Sözcükler: *Vitamin D bağlayan protein, Vitamin D, Tip1 diyabet, Adölesan*

INTRODUCTION

Type 1 diabetes (T1DM) cases are more prone to vitamin D deficiency (1, 2). Vitamin D deficiency has been reported to be associated with cardiovascular diseases, cancer, and autoimmune diseases, given that this deficiency becomes more important in T1DM (3, 4). The mechanisms explaining the relationship between T1DM, and vitamin D deficiency are not clear.

Serum 25OHD level is measured to estimate vitamin D status. This parameter has a long half-life, easier measurement, and stable serum levels. Up to 85% of circulating 25OHD is strongly bound to vitamin D binding protein (VDBP), 10–15% is weakly bound to albumin, and less than 1% circulates in the free form in the blood (5). VDBP extends the half-life of 25OHD, which is highly lipophilic in serum, allowing it to reach peripheral tissues and serve as a depot for vitamin D. According to the free hormone hypothesis, only hormones released from binding proteins can enter the cell to show its biological effects (6, 7). A change in serum VDBP level naturally affects serum 25OHD level. This effect is similar to the other transporters, such as sex hormone carrier globulin (SHBG) and thyroxine-binding protein, which change the serum concentration of the hormone they carry (8).

While serum 25OHD measurement has been used in studies investigating T1DM and vitamin D levels, VDBP has been less focused on. In a few studies investigating the cause of low vitamin D in T1DM, the focus was on VDBP levels lost in the urine, and studies are showing that this loss affects serum 25OHD levels as well as studies showing that there is no such effect (9, 10). It is unclear how VDBP changes in T1DM and its relationship with blood glucose regulation and diabetes-related factors (disease duration, insulin dose amount).

The primary aim of this study is to investigate how serum VDBP levels change in T1DM. The secondary aim is to investigate diabetes-related parameters that affect serum VDBP levels. The results obtained may provide new information about the cause-and-effect relationship between T1DM, 25OHD, and VDBP.

MATERIALS and METHODS

This study was conducted at Çanakkale Onsekiz Mart University Health Practice and Research Hospital, a tertiary referral hospital in the Southern Marmara region of Türkiye. The study group consisted of children and adolescents between the ages of 11-17 diagnosed with T1DM who were followed up in the Pediatric Endocrinology Clinic, and a control group consisting of healthy individuals in the same age group who applied to the General Pediatrics and Adolescent Clinics. The study was conducted considering seasonal effects on vitamin D levels, and all cases were included in the study between September 2020 and November 2020. Exclusion criteria included being outside the specified age limits, having a disease other than diabetes, having systemic health problems for the control group, and taking vitamin D supplements in the last two months. Duration of diabetes and insulin doses used by diabetes were recorded from the patient follow-up file.

Anthropometric Measurements

Demographic information, height, weight, body mass index, and puberty status of the participants were recorded. Growth curves specific to Turkish children were used as height and weight percentiles reference. All measurements were performed using the same method and by the same person to ensure standardization.

Laboratory Methods

The participants' blood samples were collected from the forearm vein between 7:30 and 9:30 a.m after 8 hours of fasting. Calcium, phosphorus, creatinine, and ALP were analyzed using calorimetric methods, and parathyroid hormone (PTH) was analyzed using electrochemiluminescence methods. For vitamin D level, 25OHD competitive inhibition enzyme immunoassay technique, HPLC method for Hb A1c, and ELISA method for VDBP (Elabscience, Houston, USA) were used.

Statistical Analysis

The data conformity to normal distribution was tested with the Shapiro-Wilk test, the student t-test was used to compare the normally distributed characteristics in diabetes and control groups, and the Mann-Whitney U test was used to compare the non-normally distributed characteristics between the groups. Descriptive statistics of numerical variables were given as mean and standard deviation or median (interquartile range) values. Categorical data such as gender and puberty of the study participants were presented as frequencies and percentages. The relationships between numerical variables were tested with the Spearman correlation coefficient. Univariate regression analyses were initially conducted to evaluate factors potentially affecting serum VDBP levels. The selection of relevant predictors was based on factors demonstrated in the current literature to influence VDBP levels. Variables with $p<0.25$ in univariate analysis were considered for inclusion in multiple linear regression analysis. Statistical analyses were performed using SPSS software version 24.0 for Windows. Data visualization was generated using Python programming scripts using Seaborn, Matplotlib, and Pandas libraries. $P<0.05$ was considered statistically significant.

Sample Size

We calculated the sample size of our study to reach the primary outcome. In the studies of Blanton and Thraikill, a change in serum VDBP levels was observed in a certain direction (10,11). From this, we determined that a one-tailed test was appropriate for our analysis. Using G*Power software, we conducted a power analysis to achieve the desired power of 0.80, with a medium expected effect size (Cohen's d=0.5) and an alpha level of 0.05. The calculated sample size required to achieve this power was 51 subjects per group.

RESULTS

The study included 89 patients, 40 of whom had T1DM. The mean age was 12.04±3.29 years, 59.6% (n=53) were female, and most were pubertal (68.5%). The mean BMI z-score was 0.27±1.26. In the entire group, mean Hb A1c was 7.06±2.25, serum 25OHD was 18.02±7.74 ng/ml, and approximately 41.6% (n=37) had sufficient vitamin D levels (>20ng/ml). The mean concentration of VDBP was 413.73±168.1 ug/ml (Table 1).

Comparative Analysis Between Diabetes and Control Groups

Gender distribution and pubertal status were similar between T1DM and control groups (gender p=0.91, puberty status p=0.76). Mean ages were 11.88±3.82 years for the T1DM group and 12.18±2.83 years for the control group with no significant difference (p=0.676). BMI z-scores were also not significantly different between T1DM and control groups (0.59±1.08 and 0.62±1.07, respectively) (p=0.91) (Table 2).

Biochemically, serum 25OHD levels were similar in the T1DM group and control group [median: 17.03 (12.89-22.08) and 17.62 (11.68-24.48), respectively] (p=0.701). However, VDBP levels were significantly lower in the T1DM group (median 335 µg/ml, IQR: 199.8- 517.2 µg/ml) compared to the control group (median 471.2 µg/ml, IQR: 368.3 - 533.2 µg/ml, $p<0.015$). The chi-square test results comparing vitamin D sufficiency between T1DM, and the control group showed no significant difference ($X^2= 0.5$, p=0.401) (Table 2).

Table 1: Baseline characteristics and vitamin D status of the study cohort.

Variables	Values(n=89)
Gender (Female) n, (%)	53 (59.6)
Puberty status (pubertal) n, (%)	61 (68.5)
Group (Diabetes) n, (%)	40 (44.9)
Vitamin D sufficient (>20ng/ml)	37 (41.6)
Age (years)	12.04±3.29
Height z-score	0.09 (1.48)
Weight z-score	0.33 (1.59)
BMI z-score	0.22 (1.94)
HbA1c (%)	7.06±2.25
25OHD (ng/ml)	17.03 (11.64)
PTH (pg/ml)	30.97 (18.67)
VDBP (ug/ml)	413.73±168.15

Values are presented as median (IQR) and mean±SD. **IQR:** Interquartile range, **SD:** Standard deviation, **PTH,** Parathyroid hormone, **VDBP:** Vitamin D-binding protein, **25OHD:** 25-hydroxyvitamin D, **BMI:** Body mass index. Height, Weight, and BMI are presented as z-scores adjusted for age and gender, calculated based on Turkish child growth standards.

Investigation of Factors Affecting VDBP Levels in the Entire Group and Diabetes Subgroup

Correlation analysis between HbA1c and VDBP levels in the entire group was significant (r=-0.28, p=0.007). In the diabetes and control subgroups, there was no significant correlation between HbA1c and serum VDBP (r=-0.140, p=0.37 and r=0.12, p=0.43, respectively) (Figure 1).

When investigating the factors affecting VDBP levels in the entire cohort, univariate regression analysis was performed for age, gender, BMI z-score, 25OHD, and PTH, and none of these predictors was found to be a significant predictor of serum VDBP levels. However, the presence of diabetes emerged as a significant predictor (B=87.236, SE=34.802, p=0.014). This finding persisted with multiple linear regression analysis (B=87.918, SE=34.716, p=0.013) (Table 3).

In the subgroup analysis of individuals with diabetes, possible predictors such as age, gender, BMI z-score, serum 25OHD, PTH, disease duration, HbA1c levels, and insulin dose per kilogram were evaluated in the univariate analysis. However, disease duration, 25OHD, and PTH levels had p-values below the 0.25 threshold, suggesting a potential influence on VDBP levels. However, multiple linear regression analysis indicated that none of these factors, including disease duration (B=14.743, SE=10.307, p=0.151),

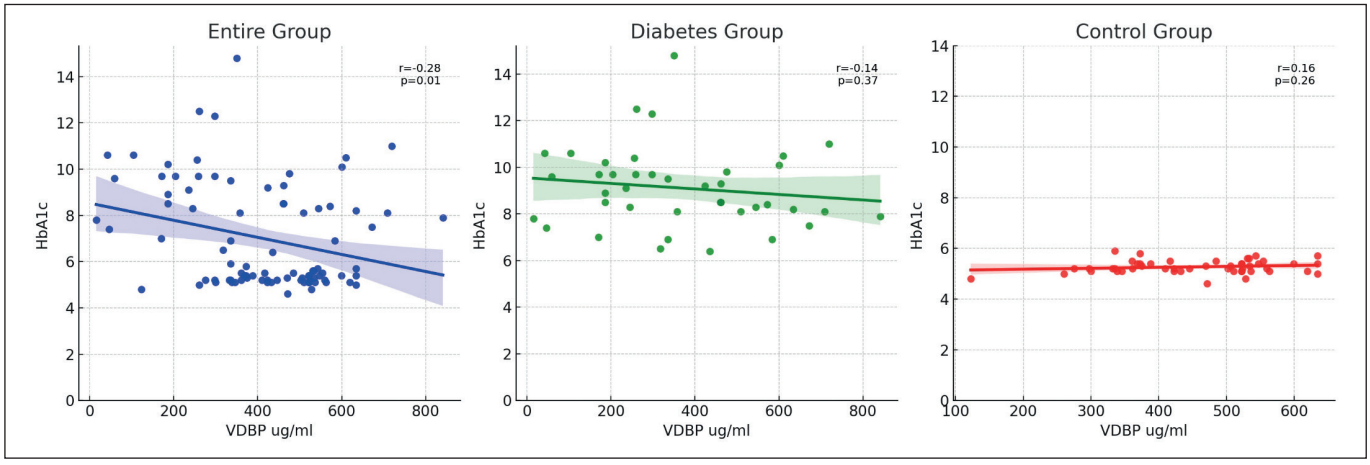

Figure 1. Correlation between Vitamin D Binding Protein (VDBP) and HbA1c across the entire cohort and separately within diabetes and control groups. The correlation coefficient (r) and p-values indicate the strength and significance of the linear relationship in each subgroup.

Table 2: Comparative analysis of demographics and biochemical markers between groups.

Variables	Diabetes (n=40)	Control (n =49)	p
Gender (female)	21 (52.5%)	32 (60.4%)	0.157
Pubertal status (puberty)	13 (46.43%)	15 (53.57%)	0.514
Age (years)	11.88±3.82	12.18±2.83	0.676
Calcium (mg/dl)	9.84 (9.58-10.07)	9.63 (9.30-10.07)	0.097
Phosphorus (mg/dl)	4.17 (3.79-4.62)	4.44 (4.09-4.96)	0.049
Albumin (gr/dl)	4.58 (4.43-4.79)	4.79 (4.48-5.00)	0.086
ALP (IU/L)	248.50 (176.75-319.50)	155.00 (92.00-263.00)	0.002
Creatinine (mg/dl)	0.56 (0.48-0.66)	0.56 (0.47-0.64)	0.830
HbA1c (%)	9.00 (8.10-9.88)	5.20 (5.10-5.40)	< 0.001
PTH (pg/ml)	28.12 (23.27-36.73)	31.88 (25.27-44.58)	0.085
VDBP ug/ml	335.00 (199.78-517.90)	471.20 (368.30-533.20)	0.015
25OHD (ng/ml)	17.03 (12.89-22.08)	17.62 (11.68-24.48)	0.701
Vitamin D sufficient	15 (37.5%)	22 (44.9%)	0.401

Values are presented as median (IQR) and mean±SD. **IQR:** Interquartile range, **SD:** Standard deviation, **PTH,** Parathyroid hormone, **VDBP:** Vitamin D-binding protein, **25OHD:** 25-hydroxyvitamin D, **BMI:** Body mass index. Height, Weight, and BMI are presented as z-scores adjusted for age and gender, calculated based on Turkish child growth standards. p < 0,05.

Table 3: Univariate and multivariate linear regression analysis for serum VDBP levels in the entire group.

	Univariate analysis			Multiple linear regression analysis		
Variable	**B**	**SE**	**P-value**	**B**	**SE**	**p**
Age (years)	3.223	5.462	0.557	-		-
Gender	-36.613	36.313	0.316	-		-
BMI z-score	16.011	14.172	0.242	16.578	13.752	0.231
Diabetes status (Group)	87.236	34.802	0.014	87.918	34.716	0.013
25(OH)D (ng/ml)	-0.294	2.329	0.9	-		-
PTH (pg/ml)	0.856	1.166	0.465	-		-

Dependent Variable: Serum VDBP ug/ml. Coefficients (B) and Standard Errors (SE) are provided for each predictor variable. In the univariate analysis, variables with a p-value < 0.25 were included in the subsequent multiple linear regression analysis. **PTH:** Parathyroid hormone, **BMI:** Body Mass Index, **VDBP:** Vitamin D binding protein.

Table 4: Univariate and multiple linear regression analyses of factors influencing serum VDBP levels in the diabetes group.

	Univariate analysis			Multiple linear regression analysis		
Variable	**B**	**SE**	**P-value**	**B**	**SE**	**p**
Gender (female)	-27.063	67.047	0.689	-		-
Disease duration	13.850	10.214	0.183	14.743	10.307	0.151
Age (years)	-1.800	8.896	0.848	-		-
Total insulin dosage per kg	112.779	127.517	0.382	-		-
BMI z-score	-21.446	32.918	0.519	-		-
HbA1c	-17.7530	19.660	0.372	-		-
25OHD (ng/ml)	-6.312	4.913	0.207	-5.916	4.800	0.226
PTH (pg/ml)	3.363	2.548	0.195	3.607	2.500	0.158

Dependent Variable: Serum VDBP ug/ml. Coefficients (B) and Standard Errors (SE) are provided for each predictor variable. In the univariate analysis, variables with a p-value <0.25 were included in the subsequent multiple linear regression analysis. **PTH:** Parathyroid hormone, **BMI:** Body Mass Index, **VDBP:** Vitamin D binding protein.

25OHD (B=-5.916, SE=4.800, p=0.226), and PTH (B=3.607, SE=2.500, p=0.158), were significant predictors of serum VDBP levels (Table 4).

DISCUSSION

In this study, serum VDBP levels were found to be significantly lower in T1DM cases, but serum 25OHD levels did not differ between groups. The presence of diabetes was the only factor affecting serum VDBP levels in the entire group. However, in the T1DM subgroup, HbA1c, diabetes duration, and insulin dose were not effective factors on VDBP levels. These findings suggest that diabetes may influence VDBP metabolism or distribution through mechanisms that are not directly related to metabolic control.

Many studies report that 25OHD levels are lower and vitamin D deficiency is higher in T1DM. However, we did not find any difference between the groups. This unexpected result is in line with some studies in the literature (12). Kim et al. reported that the high prevalence of vitamin D deficiency may have masked the emergence of a difference in the cohort in which they examined diabetic patients without microalbuminuria (9). Similar results were also obtained in the study by Medina et al. (13). These differences in results may be due to ethnic differences in the cohorts, laboratory measurement methods, and differences in the distribution of genetic determinants for vitamin D (GC, VDR, etc.). However, in line with Kim et al.'s interpretation, we think that in our study, the expected difference between the groups did not emerge due to the geography where vitamin D deficiency is common.

In a cohort including 203 T1DM and 153 controls, serum VDBP levels were ~10% lower in T1DM cases in the Blanton et al. study (11). A similar result was found by Thraikill et al., who attributed this change to increased VDBP loss with urine (10). Choe Y et al. found lower levels of 25OHD and VDBP in T1DM patients (14). Despite these studies, information on VDBP change in T1DM is contradictory. Cave et al. observed a positive correlation between T1DM and VDBP in a survey including T1DM in an African population (15). The researchers discussed whether the increase

in VDBP was causal or a result of T1DM. In the study by Kim et al., 25OHD and serum VDBP did not differ in the T1DM group (9).

Notably, the risk factor affecting VDBP level in the whole study group was diabetes status. However, multivariant regression analysis performed in the diabetes group showed that the factors affecting VDBP level were not HbA1c, duration of diabetes, or insulin dose used. This suggests that unmeasured factors caused by diabetes independent of blood glucose control may affect VDBP. These factors may be due to inflammation, renal excretion, and differences in hepatic synthesis processes.

A negative relationship between VDBP and insulin has been reported (16, 17). Therefore, the effect of insulin doses on VDBP levels and vitamin D levels is of interest. In the study of Tunç et al., 100 children with diabetes were examined. They reported an inverse correlation between daily insulin dose and vitamin D levels (18). Bae et al. said that vitamin D deficiency and insufficiency were high in type 1 DM patients, but this was not associated with diabetes duration, HbA1c, and daily insulin dose (1). In the study by Setayesh et al., no correlation was observed between insulin and 25OHD, but a negative correlation between VDBP and insulin was noted (17). In light of these studies, another possible factor affecting VDBP levels may be the insulin doses used. However, in our study, daily insulin doses were not correlated with VDBP.

Blanton et. al found serum VDBP levels to be lowest in T1DM cases and highest in healthy controls. Interestingly, moderately low VDBP was found in first-degree healthy relatives of T1DM cases. (11). In addition, VDBP levels were not related to HbA1c, diabetes duration, or age in the diabetes group. As a result, it shows that genetic determinants have a significant effect on serum VDBP level in T1DM, beyond metabolic variations and blood glucose control. GC gene, which encoded VDBP, polymorphisms have been reported to affect serum VDBP and serum 25OHD levels (8). Especially in the GC2 phenotype, which is associated with minor allele change in rs4588 (19), VDBP levels tend to be approximately 10% lower (5).

We do not know the disruption of these polymorphisms in our cohort, given that we cannot exclude the effect of the GC polymorphisms. Another possible mechanism is renal excretion of VDBP, an albumin-like protein. VDBP is correlated with proteinuria in diabetic patients with nephropathy (20). However, the effect of this excretion on serum VDBP levels is not clear (8). Kim et al. reported that VDBP excretion did not affect serum levels in patients without albuminuria in T1DM, but the cohort was small (9). Thrailkill et al. emphasized that urinary VDBP excretion may affect serum VDBP (10). VDBP passes to glomerular filtrate and is reabsorbed from the apical surface via megalin. Disruptions in this reuptake process have been shown to cause decreases in serum VDBP and serum 25OHD. However, the fact that urinary VDBP levels were not measured in our study prevents us from excluding this possible cause.

Limitations and Future Studies

Some limitations are taken into account when concluding this study's results. First of all, the sample size may limit the generalizability of the obtained results. However, when evaluated together with the few studies in the literature, it provides results reflecting VDBP changes in T1DM. A notable constraint was the limited time frame for patient recruitment, which directly impacted our sample size and affected the power of the study. We opted to adhere to this limited time to homogenize the effects of sunlight exposure on vitamin D levels. Our other limitation is that dietary factors were not determined. In addition, failure to examine GC polymorphism distribution and urine VDBP excretion resulted in limitations in understanding the mechanisms behind our results.

Future studies are needed with a larger cohort, examining VDBP excretions, GC polymorphism distribution, and dietary factors. These studies will provide valuable information about the cause-and-effect relationship between diabetes and vitamin D.

CONCLUSION

In conclusion, this study shows that serum VDBP levels are considerably lower in the T1DM cohort. It seems that serum 25OHD, glycemic control, disease duration, and insulin doses do not affect the change in serum VDBP levels in diabetic patients. Other factors beyond these may affect VDBP. Large-scale studies investigating the cause-and-effect relationship of the difference in serum VDBP in diabetes may pave the way for newer approaches to managing vitamin D homeostasis.

Acknowledgment
None

Author Contributions
Eda Gül Özcan, Durmuş Doğan contributed to the conception and design of the study. **Eda Gül Özcan** prepared the manuscript and analyzed the data, **Durmuş Doğan** analysis, review & editing. All authors contributed to the article and approved the submitted version.

Conflict of Interest
The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. A part of this study was presented

as an oral presentation at the 18th Uludağ Pediatrics Winter Congress (2022), Bursa, Turkiye 2022.

Funding
It was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit as a project numbered TTU-2021-3613.

Ethical Approve
This study was approved by Çanakkale Onsekiz Mart University Clinical Research Ethics Committee with the decision numbered 2011-KAEK-27/2020-E.2000099549, dated 26.08.2020.

Peer Review Process
Extremely peer-reviewed and accepted.

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