



Free and Bioavailable Vitamin D Levels of Patients with Type 1 Diabetes Mellitus and Association with Bone Metabolism

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Abstract

Aim Vitamin D deficiency is known to be associated with metabolic bone diseases. The aim of this study is to evaluate vitamin D and calculated free and bioactive vitamin D levels of type 1 diabetic patients and to evaluate the association with bone turnover markers.

Method This cross-sectional study includes 60 patients admitted to endocrinology outpatient clinic with diagnosis of type 1 diabetes mellitus and 60 controls. Weight, height and waist circumference were recorded and blood samples were taken for measurement of 25-hydroxyvitamin D (25(OH)D), vitamin D binding protein (VDBP), osteocalcin, bone alkaline phosphatase (bone-ALP), c-telopeptide. Free and bioavailable vitamin D levels were calculated with formula.

Results Vitamin D levels of type 1 diabetic patients were significantly higher ($p=0.01$). Parathormone levels of the group with vitamin D level under 20 ng/ml was significantly higher ($p=0.029$). VDBP levels were similar in both groups. Correlation analysis of free and bioavailable vitamin D level with osteocalcin, c-telopeptide, bone alkaline phosphatase revealed only a weak significant correlation between free vitamin D and osteocalcin ($r=-0.201$; $p=0.028$). A negative correlation was determined between 25(OH)D and parathormone levels ($r=-0.294$; $p<0.005$). Serum osteocalcin, bone alkaline phosphatase and c-telopeptide levels of control group were significantly higher.

Conclusion 25(OH)D levels of the study population was extremely low. The measurement of VDBP and calculated free and bioactive vitamin D levels did not show a better correlation with bone turnover markers according to 25(OH)D levels.

Keywords VDBP · Free vitamin D · Bioavailable vitamin D · Type 1 DM

Introduction

Type 1 Diabetes Mellitus (DM) is an immune associated disease caused by destruction of insulin secreting pancreatic beta cells. It is known to be a childhood or youth disease but can be diagnosed at any age [1]. Genetic, autoimmune, and environmental factors are effective in etiology. In genetically predisposed patients, environmental factors may trigger the disease [1].

In ecologic studies, the incidence of type 1 DM was found to be higher in population that exposed to lower ultraviolet-B (UV-B) beam. In studies that investigate the relationship between vitamin D and type 1 DM; 25-hydroxyvitamin D (25(OH)D) levels were found to be lower in type 1 DM group [2]. These patients have lower bone mineral density and higher fracture risk compared to healthy population [3].

Vitamin D is synthesized from previtamin D with the effect of UV-B in the skin, by degradation of carbon bonds between 9 and 10th carbons of 7-dehydrocholesterol [4]. By

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1-alpha hydroxylase it is activated in kidneys. Vitamin D receptors are found in many tissues like osteoblasts, mononuclear cells, lymphocytes, heart muscle, colon, brain, pituitary gland, thyroid, breast, liver, kidneys, skin, prostate gland, gonads and pancreas islet cells [4]. Vitamin D deficiency leads to decrease in intestinal calcium absorption, hyperparathyroidism, decrease in bone mineral densitometry and increase in fracture risk. In recent studies bioactive and free vitamin D were shown to be better associated with bone metabolism in some studies [5, 6].

Vitamin D Binding Protein (VDBP) is a polymorphic, one strand serum glycoprotein. In circulation 90% of 25(OH) D is bound to VDBP and 10% bound to albumin, less than 1% is in free form. Biologically active vitamin D is the free vitamin D. VDBP polymorphisms and ethnic and genetic features affect the VDBP levels. VDBP is the most polymorphic gene with over 120 described variants. Gc1F, Gc1S and Gc2 are the most common variants and different variants have different affinities for 25(OH)D [7]. Also, affinities seem to be different in different clinical conditions as in third trimester of pregnancy, total vitamin D levels and VDBP levels tend to be higher [8]. Also in patients with liver disease, directly measured free vitamin D levels tend to be higher [8]. Allelic variation may also affect the affinity as Gc2 variants have a modest reduction in levels of 25(OH)D [9]. In a study from United States of America (USA) in black race 25(OH) D levels were found to be lower than white race but bioactive vitamin D level calculated with VDBP levels found to be similar with white race [5]. Mathematical methods to calculate bioactive and free vitamin D by using VDBP and albumin levels correlated well with direct measurements in studies [10]

The aim of this present study is to evaluate free and bioavailable vitamin D levels by calculating with the formula using VDBP and albumin levels of type 1 diabetic patients and to evaluate its relationship with bone mineral metabolism.

Material and Method

This cross-sectional study was conducted in Marmara University Hospital Endocrinology outpatient clinic after approval by the local Ethics Committee (Protocol number: 09.2015.098). Volunteer participants were included after written consent was signed. Financial resources were provided by Marmara University Scientific Research Projects Coordination Unit (SAG-C-TUP-080715). Sixty healthy volunteers and sixty patients with type 1 DM according to American Diabetes Association guidelines [11], were included in this study. The participants had no chronic inflammatory disease, the liver and renal functions were normal and over

the last 6 months they did not have vitamin D replacement. Socio-demographic characteristics were recorded, weight, height and waist circumference were measured, information about complications were obtained from the medical records. Thyroid Stimulating Hormone (TSH), creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), High density lipoprotein cholesterol (HDL-C), triglyceride, total cholesterol, fasting glucose levels of the last visit within 6 months were recorded. HbA1c levels over the last 3 months were recorded. Retinopathy examination over the last 6 months was recorded and spot urine microalbumin/creatinine ratio was recorded. Eight hours fasting blood was taken from the brachial vein to measure serum osteocalcin, c-telopeptide (C-TX), bone alkaline phosphatase (Bone-ALP), calcium, inorganic phosphate, parathormone, 25(OH)D, VDBP levels. Sample collection was done between December-March. All blood samples were stored at -20 °C after centrifugation and biochemical measurements were done by Marmara University Biochemistry department.

Serum 25(OH)D levels were measured from samples with electrochemiluminescence immunologic test ECLIA at Modular Analytics E170 immunologic test autoanalyzer (Roche, Germany). Plasma 25(OH)D level measurement range was 7.5–175 nmol/L or 3–70 ng/ml.

Serum calcium level was measured by photometric color test with quantitative technic (Beckman Coulter, USA). Reference range was reported as 4–20 mg/dl.

Serum inorganic phosphate level was measured by photometric UV test by quantitative technic (Beckman Coulter, USA). The reference range was reported as 1–20 mg/dl.

Parathormone level was measured by access immunoassay system (Beckman Coulter, USA). Reference range was reported as 12–88 pg/ml.

VDBP level was measured by sandwich enzyme immunoanalysis method (R&D systems, USA). Samples were diluted 2000 times. Measurement range was found 0.15–3.74 ng/ml. Coefficient of variation was 5.7–6.2% and coefficient of variation between studies was 5.1–7.4%.

Serum osteocalcin was measured by Enzyme Linked Immunosorbent Assay (ELISA) method (Sunred, Shanghai, China). Measurement range was 0.5–150 ng/ml.

Bone alkaline phosphatase was measured by sandwich ELISA method (Sunred, Shanghai, China). Measurement range was 3–900 U/L.

Serum C-TX was measured by sandwich ELISA method (Sunred, Shanghai, China). Measurement range was 0.5–150 ng/ml.

Free and bioavailable vitamin D levels of the patients were calculated with formula by using the measured albumin, vitamin D binding protein, 25(OH) D levels [10].

Formula: Correlation coefficient between measurement with ultrafiltration method and calculated 25(OH)D is 0,925 [10].

$$(K(\text{albumin}) : 6 \times 10^5, K(D - \text{bindingprotein}) : 7 \times 10^8)$$

$$\text{Free25(OH)D} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

$$\begin{aligned} \text{Bioactive25(OH)D} &= (D_{\text{free}}) + (D_{\text{Albumin}}) \\ &= [K(\text{albumin}) * [\text{albumin}] +] * (D_{\text{free}}) \end{aligned}$$

$$\begin{aligned} a &= K(D - \text{bindingprotein}) * K(\text{albumin}) * [\text{albumin}] \\ &+ K(D - \text{bindingprotein}) \end{aligned}$$

$$b = K(D - \text{bindingprotein}) * [D - \text{bindingprotein}]$$

$$K(D - \text{bindingprotein}) * [DTOTAL] + K(\text{albumin}) * [\text{albumin}] + 1$$

$$c = -[DTOTAL]$$

Statistical analysis were done by SPSS version 17 program. Normality analysis was done by Kromogrow Smirnow test. For the normally distributed data, independent group t-tests and Pearson correlation tests were performed. Multivariate regression analysis was done for parameters that have significant correlations. For the data that does not distribute normally, logarithmic transformation was done. Kruskal Wallis test was used as nonparametric test. $p < 0.05$ was considered as statistically significant.

Results

Baseline characteristics

A group of sixty patients with type 1 diabetes (F/M: 31/29) and 60 healthy subjects (F/M: 31/29) who met the criteria and who were sex-matched were included in this study. Mean age, BMI, waist circumference and education status were similar between groups (Table 1).

Laboratory parameters

Liver and renal function tests of the participants were in normal range. Mean duration of illness in type 1 DM group was 10.33 ± 6.88 years, 28.3% of the patients had retinopathy and 25% had nephropathy. Mean HbA1c was $8.43 \pm 2.07\%$. In both groups; age, Body Mass Index (BMI), waist circumference, glucose, TSH, HDL-C, Low density Lipoprotein

Table 1 Demographic parameters and baseline characteristics of patients and control group

	Type 1 DM	Control	P value
Sex (F/M)	31/29	31/29	0.999
Age (years)	29.5 ± 7.55	28.5 ± 5.58	0.389
BMI (kg/m ²)	24.35 ± 3.62	24.98 ± 4.13	0.374
Waist circumference (cm)	79.76 ± 9.81	80.28 ± 9.53	0.770
Disease duration (years)	10.33 ± 6.88	-	-
Retinopathy (±)	17/43	-	-
Nephropathy (±)	15/45	-	-

cholesterol (LDL-C), VDBP, parathormone, calcium, inorganic phosphate, albumin levels had normal distribution.

There were no significant difference in HDL-C, LDL-C, PTH, VDBP levels of groups. There was a statistically significant difference of TSH levels of type 1 diabetic patients and control group but all of the patients were euthyroid. Serum calcium level of patients with type 1 DM was lower and inorganic phosphate of patients with type 1 DM was higher according to control group but all of the patients had normal calcium and phosphor levels. Laboratory parameters of the patients with and without diabetes were shown in Table 2.

All of the patients were cathegorised according to vitamin D levels, the parathormone level of group with 25(OH)D < 20 ng/ml was 44.05 ± 19.41 pg/ml, parahormone level of the group with 25(OH)D ≥ 20 ng/ml was 35.95 ± 14.89 pg/ml and there was a significant difference ($p = 0.029$) When patients were grouped as 25(OH)D < 20 ng/ml and 25(OH)D ≥ 20 ng/ml, the 63.3% of the patients with type 1 DM and 78.3% of the control group were vitamin D deficient ($p = 0.121$). Ten % of the patients with type 1 DM and 6.6% of the control group had 25(OH)D > 30 ng/ml. In patients with type 1 DM serum LDL-C of the patients with 25(OH)D < 20 ng/ml was significantly higher ($p = 0.015$). In control group, the albumin ($p = 0.023$), parathormone ($p = 0.013$) and calcium ($p = 0.001$) were significantly higher. There was a significant difference after adjustment of calcium levels according to albumin levels. The laboratory parameters of the patients and control group with vitamin D < 20 ng/ml and vitamin D ≥ 20 ng/ml were shown in Table 2.

Free and bioactive vitamin D levels and association with bone metabolism

Serum 25(OH)D ($p = 0.010$) and free vitamin D ($p = 0.027$) levels of the type 1 DM group was higher than the control group (Table 3). Bioactive vitamin D levels were higher in type 1 DM group but this difference was not significant. 25(OH)D, bioactive and free vitamin D levels of type 1 DM

Table 2 Biochemical parameters of patients and the control group

	Type 1 DM (n:60)		Control (n:60)		p value
	25(OH)D < 20 (n = 38)	25(OH)D ≥ 20 (n = 22)	25(OH)D < 20 (n = 47)	25(OH)D ≥ 20 (n = 13)	
Glucose (mg/dl)	193.15 ± 103.71	191.04 ± 81.29	89.41 ± 10.62	86.15 ± 10.43	< 0.001 [#]
TSH (mIU/ml)	1.92 ± 1.074	2.12 ± 1.28	1.55 ± 0.600	1.41 ± 0.79	0.005 [#]
HDL-C (mg/dl)	53.57 ± 13.64	58.81 ± 10.57	51.41 ± 8.87	50.84 ± 12.03	> 0.05
LDL-C (mg/dl)	125.47 ± 36.14	101.68 ± 32.84	125.9 ± 32.2	111.15 ± 31.56	0.015*
PTH (pg/ml)	40.98 ± 16.36	38.97 ± 16.70	46.6 ± 21.46	30.83 ± 9.72	0.013**
Ca (mg/dl)	9.60 ± 0.502	9.44 ± 0.392	9.95 ± 0.41	9.507 ± 0.398	0.001 [#]
P (mg/dl)	3.46 ± 0.595	3.64 ± 0.649	3.29 ± 0.45	3.41 ± 0.570	0.046 [#]
VDBP (ng/ml)	347.2 ± 230.5	331.4 ± 254.7	365.7 ± 219.1	386.4 ± 288.9	> 0.05
Albumin (g/dl)	4.42 ± 0.389	4.34 ± 0.288	4.78 ± 0.373	4.51 ± 0.304	< 0.001 [#]

* p value of the comparison of the LDL-C levels of diabetic patients with 25(OH)D < 20 ng/ml and 25(OH)D > 20 ng/ml

** p value of the comparison of parathormone levels of control group with 25(OH)D < 20 ng/ml and 25(OH)D > 20 ng/ml

[#] comparison of the control group and type 1 DM

Table 3 25(OH)D, free, bioactive vitamin D levels and bone turnover markers of patients and control group

	Type 1 DM N = 60		Control N = 60		p value
	25(OH)D < 20 (N = 38)	25(OH)D ≥ 20 (N = 22)	25(OH)D < 20 (N = 47)	25(OH)D ≥ 20 (N = 13)	
25(OH)D (ng/ml)	16.270 (3–43.89)		10.620 (3.01–42.50)		0.01
Free vitamin D (pg/ml)	4.0215 (0.258–21.719)		2.55 (0.512–21.237)		0.27
Bioactive vitamin D (ng/ml)	1.574 (0.258–21.719)		1.024 (0.198–8.448)		> 0.05
Bone ALP (U/L)	247.59 (148.8–667.7)	216.55 (116.2–684.7)	364.32 (130.4–928.2)	330.6 (126.5–900.7)	0.015*
Osteocalcin (ng/ml)	39.59 (22.08–119.01)	31.05 (22.24–122.59)	87.92 (27.51–172.3)	88.71 (27.51–172.3)	< 0.001**
C-telopeptide (ng/ml)	22.25 (11.33–128.36)	20.84 (11.01–130.44)	67.124 (16.23–159.5)	73.49 (22.33–155.04)	< 0.001 [#]

* significant difference of bone ALP levels of patients with diabetes and controls, ** significant difference of osteocalcin levels of diabetic patients and controls, # significant difference of c-tx levels of patients with diabetes and controls

patients with and without retinopathy and nephropathy were similar ($p > 0.05$).

Bone alkaline phosphatase ($p = 0.015$), osteocalcin ($p < 0.001$) and c-telopeptide ($p < 0.001$) levels were higher in the control group (Table 3). There was no statistically significant difference in levels of bone ALP, osteocalcin and c-telopeptide between the group with 25(OH)D < 20 ng/dl and 25(OH)D > 20 ng/dl. VDBP levels of type 1 DM group and control group were similar, there was no difference between both sex groups. Also VDBP levels of the group with 25(OH)D < 20 and 25(OH)D > 20 were similar. Laboratory parameters were shown in Table 3.

Regression and correlation analysis

When all of the participants were included in statistical analysis, BMI had positive correlation with waist circumference ($\rho = 0.835$, $p < 0.001$), LDL-C levels ($\rho = 0.197$,

$p = 0.033$), parathormone levels ($\rho = 0.236$, $p = 0.01$) and negative correlation with HDL-C levels ($\rho = -0.253$, $p = 0.005$), osteocalcin levels ($\rho = -0.239$, $p = 0.009$) and 25(OH)D levels ($\rho = -0.240$, $p = 0.008$). There was positive correlation between waist circumference and LDL-C ($\rho = -0.218$, $p = 0.018$) and negative correlation with HDL-C ($\rho = -0.360$, $p < 0.001$) and osteocalcin ($\rho = -0.217$, $p = 0.017$).

There was a negative correlation between age and osteocalcin (type 1 DM $r = -0.506$, control $r = -0.350$) c-telopeptide (type 1 DM $r = -0.544$, control $r = -0.297$) and bone alkaline phosphatase (type 1 DM $r = -0.534$, control $r = -0.278$) in both groups. There was a positive correlation between age and 25(OH)D ($\rho = 0.232$, $p = 0.011$).

Osteocalcin, c-telopeptide and bone alkaline phosphatase increased with decrease in 25(OH)D, free and bioavailable vitamin D but there was only weak significant correlation between free vitamin D and osteocalcin ($r = -0.201$, $p = 0.028$) (Table 4). Multivariate regression analysis was

Table 4 Correlation analysis of vitamin D levels and bone turnover parameters

Study population		25(OH)D	ALP (U/L)	OC (ng/ml)	CTX (ng/ml)	Free vitamin D (pg/ml)	Bioactive vitamin D (ng/ml)	PTH(pg/ml)
25(OH)D	Pearson	1	-0.11	-0.156	-0.126	0.694	0.697	-0.294
	p	-	0.214	0.09	0.171	<0.001	<0.001	0.001
ALP (U/L)	Pearson	-0.11	1	0.873	0.891	-0.171	-0.155	-0.122
	p	0.214	-	<0.001	<0.001	0.062	0.092	0.184
OC (ng/ml)	Pearson	-0.156	0.873	1	0.930	-0.201	-0.176	-0.021
	p	0.09	<0.001	-	<0.001	0.028	0.055	0.823
CTX (ng/ml)	Pearson	-0.126	0.891	0.930	1	-0.163	-0.138	-0.028
	p	0.171	<0.001	<0.001	-	0.075	0.132	0.763
Free vitamin D (pg/ml)	Pearson	0.694	-0.171	-0.201	-0.163	1	0.996	-0.186
	p	<0.001	0.062	0.028	0.075	-	<0.001	0.042
Bioactive vitamin D (ng/ml)	Pearson	0.697	-0.155	-0.176	-0.138	0.996	1	-0.193
	p	<0.001	0.092	0.055	0.132	<0.001	-	0.035
PTH(pg/ml)	Pearson	-0.294	-0.122	-0.021	-0.028	-0.186	-0.193	1
	p	0.001	0.184	0.823	0.763	0.042	0.035	-

done for osteocalcin and there was a significant correlation with fasting blood glucose ($\beta = -0.41$, $p < 0.001$), age ($\beta = -0.359$, $p < 0.001$), BMI ($\beta = -0.209$, $p = 0.007$). All of the participants with and without vitamin D deficiency were evaluated, serum osteocalcin, c-telopeptide, bone-ALP levels were statistically similar between groups.

Correlation analysis was done for VDBP, there was not a significant correlation with age, BMI, waist circumference, LDL-C, osteocalcin, C-TX, bone ALP. The VDBP levels were similar between type 1 DM and control group. VDBP levels of the patients deficient of vitamin D and not were statistically similar. VDBP levels of male and female patients were similar.

There was a significant correlation between parathormone and 25(OH)D levels ($r = -0.294$, $p = 0.001$). There was a weak correlation between parathormone and bioactive ($r = -0.193$, $p = 0.035$) and free vitamin D ($r = -0.186$, $p = 0.042$) (Table 4). When the multivariate regression analysis was done as the dependent variable parathormone and the independent variables were 25(OH)D, free vitamin D, bioactive vitamin D, BMI; 25(OH)D was found to be the predictive factor for parathormone levels ($\beta = -0.294$, $p = 0.001$).

There was a significant correlation between age and osteocalcin ($\rho = -0.378$, $p < 0.001$), bone alkaline phosphatase ($\rho = -0.423$, $p < 0.001$) and C-TX ($\rho = -0.422$, $p = 0.001$).

Discussion

Vitamin D deficiency is a global problem and its relationship with many chronic diseases is currently being investigated. It is thought that VDBP levels, which is the main vitamin D carrier protein in blood may affect the level of circulating bioactive vitamin D. In studies vitamin D levels and VDBP

levels of patients with type 1 DM were shown to be lower than healthy population [12, 13]. In this recent study, the aim is to evaluate if bioactive and free vitamin D levels are a better indicator for bone metabolism than 25(OH)D in type 1 diabetic patients.

In this study only 8.3% of the participants have 25 (OH)D levels in normal range similar to other studies carried out in Turkey [14]. There are studies that claim 25 (OH)D of type 1 DM patients are lower or similar with control group [2, 14, 15]. According to the results of a meta-analysis serum 25(OH)D levels of patients with type 1 DM were found to be lower than healthy population. In another meta-analysis 25(OH)D levels were found to be lower than healthy population but this difference was not found to be significant in patients over 14 years old [2, 15]. Also in a study 25 (OH)D levels of diabetic children were found to be similar with non-diabetic children [16]. In this current study, different from the previous studies the 25(OH)D level of the patients with type 1 DM found to be higher and in regression analysis BMI and age are the determinants that affect vitamin D levels. Participants who did not have vitamin D replacement within 6 months were included in this study but the clothing styles, nutrition and UVB exposure may have affected this condition.

In this study when all the participants were evaluated, parathormone levels of the participants with 25(OH)D < 20 ng/ml was significantly higher. There was a significant negative correlation between 25(OH)D and parathormone levels, free and bioavailable vitamin D levels did not show significant correlation when evaluated with multivariate regression analysis. Also there was not a significant correlation with bone turnover markers, only free vitamin D and osteocalcin showed a statistically significant negative correlation. This study was planned with the hypothesis of free and bioavailable vitamin D may be a better indicator of bone metabolism than 25(OH)D but no evidence

was found to support this. When the previous studies were evaluated, a study enrolled in American black population, vitamin D levels were lower than the white population but their fracture risk was also lower, and in this study VDBP levels of the black population were found to be lower which might lead to measurement of 25(OH)D levels low. The calculated bioavailable vitamin D levels were found similar with white population in this study [5]. Also in a study enrolled in hemodialysis patients, parathormone levels showed better correlation with bioactive vitamin D [6]. There are studies in literature that found bioavailable and free vitamin D does not give better information about bone metabolism. In a study that evaluated the effect of vitamin D replacement on free vitamin D, bioavailable vitamin D, parathormone and bone turnover markers, free and bioactive vitamin D did not show a better correlation like this current study. [17]. In a study enrolled in healthy population, free vitamin D levels correlated better with BMD [18] but in a larger population study this correlation was not shown [14]. In a recent review about VDBP and biologic activity of vitamin D, bioavailable and free vitamin D were not shown to be a better indicator for vitamin D status [19]. Also in a recent study enrolled in elderly population; after giving 3750 IU vitamin D as treatment for a year, the calculated free and bioavailable vitamin D levels did not have a better correlation than 25(OH)D with parathormone, osteocalcin, C-TX levels and BMD levels [20].

VDBP levels of the two groups were similar and does not show any correlation with bone turnover markers. In a study enrolled in type 1 diabetics, VDBP levels of the patients with type 1 diabetes were found to be lower [12]. Evaluation in a wider population may give additional information.

Similar with the previous studies, bone turnover markers of the patients with type 1 DM were found to be lower [21, 22]. Negative correlation was found between 25(OH)D and bone turnover markers in this study but not statistically significant. The results of the studies in literature are also contradictory. In a randomized controlled study enrolled in middle aged population, vitamin D supplementation had no effect on bone turnover markers [23]. Also, in a study enrolled in postmenopausal women, a significant negative correlation was found between 25(OH)D and parathormone, serum C-telopeptide and PINP showed significant correlation in patients with 25(OH)D < 20 ng/ml and parathormone > 58 pg/ml [24].

As a result in this study, free and bioavailable vitamin D levels did not show a better correlation with parathormone levels and bone turnover markers. The results of the studies in literature are contradictory and it is difficult to say that calculated free and bioavailable vitamin D or directly measured free vitamin D might be a better factor in predicting bone metabolism [20, 25].

Limitations of this study are; the participants were not evaluated for the UVB exposure, nutrition and clothing styles. BMD levels were not evaluated. Free and vitamin D levels were calculated with a formula and not measured directly. Only 10% of type 1 diabetics and 8.4% of healthy population had 25 (OH)D

levels in normal range, including a population with normal 25 (OH)D levels may give additional information.

Conclusion

25 (OH)D levels in the study population were extremely low both in patient and control groups. Calculated free and bioavailable vitamin D levels did not have a better correlation with bone metabolism. Measuring VDBP levels together with vitamin D did not make any additional contribution to determining effect on bone. As a further research, for the evaluation of vitamin D binding affinities, VDBP polymorphisms may give additional information and measurement with a direct method may give additional information about the effect of bioavailable form of vitamin D on bone metabolism.

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Author contribution Ceyda Dincer Yazan and Oguzhan Deyneli conceptualized the study. The biochemical analysis were studied by Ali Yaman, Onder Sirikci and Goncagul Haklar. The draft was written by Ceyda Dincer Yazan and revisions were made by Oguzhan Deyneli.

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Data availability The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

The data of this study is available upon asking the corresponding author.

Declarations

Ethics approval This study was approved by the local ethic comitee.

Informed consent Written informed consent was taken from the all participants.

Conflicts of Interest The authors have declared that no conflict of interest exists.

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