A lack of association between vitamin D-binding protein and 25-hydroxyvitamin D concentrations in pediatric type 1 diabetes without microalbuminuria

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Purpose: Vitamin D deficiency is reported to be more common in type 1 diabetes patients and might be associated with the increased urinary loss of vitamin D binding protein (VDBP) consequent to impaired 25-hydroxyvitamin D (25(OH)D) circulation. We aimed to evaluate the possible increased urinary loss of VDBP, a correlation between VDBP and circulating 25(OH)D level, and risk factors influencing low vitamin D level in pediatric type 1 diabetes patients without microalbuminuria.
Methods: This is a cross-sectional study of subjects who visited Seoul National University Children's Hospital between January and March 2013. Forty-two type 1 diabetes patients and 29 healthy controls were included. Biochemical parameters including serum and urine VDBP concentrations were analyzed.
Results: There was no significant difference in the frequency of vitamin D deficiency or serum 25(OH)D level between the 2 groups. The serum and urine VDBP concentrations did not show any difference between the 2 groups. Serum 25(OH)D level did not correlate with serum or urine VDBP. Multivariate regression analysis revealed that daylight outdoor hours (β=2.948, P=0.003) and vitamin D intake (β=2.865, P=0.003) affected the 25(OH)D level; the presence of type 1 diabetes or urinary VDBP excretion was not significant.
Conclusion: In pediatric type 1 diabetes patients, urinary VDBP excretion did not contribute to low serum 25(OH)D level in the setting of normoalbuminuria. The factors associated with 25(OH)D level during winter periods were daylight outdoor hours and vitamin D intake. Further studies including both micro- and macroalbuminuria patients with type 1 diabetes are warranted.

Keywords: Vitamin D-binding protein, Ergocalciferols, Type 1 diabetes mellitus, Child, Albuminuria

Introduction

Several potential causative mechanisms of the reduced bone mineral density associated with type 1 diabetes in adults have been proposed, including bone changes due to advanced glycation, hypercalciuria associated with glycosuria, increased inflammatory cytokine levels, and a high prevalence of vitamin D deficiency[1]. Many studies have suggested that vitamin D deficiency is more common in individuals with type 1 diabetes, compared with controls[1,2], but the mechanisms that contribute to vitamin D deficiency in type 1 diabetes patients remain unclear[3]. Currently, the importance of a sufficient vitamin D status has been emphasized by the discovery of nonclassic actions of vitamin D on insulin resistance, cardiovascular diseases, cancer, and others[4,5]. 25-Hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D, is the best indicator with which to monitor vitamin D status. Approximately 88% of serum 25(OH)D is bound to the vitamin D-binding protein (VDBP), a major vitamin D metabolite

transport protein in the plasma[6]. The VDBP + 25(OH)D complex is filtered across the glomerulus and transported to the proximal tubule. Next, reabsorption of the VDBP + 25(OH)D complex by the multiligand receptors, megalin and cubilin, promotes the conversion of 25(OH)D to 1,25-dihydroxyvitamin D $(1,25(OH)_2D)$ through the activity of 1α-hydroxylase[3,7,8]. The risk of vitamin D deficiency might increase along with an increased loss of urine VDBP (uVDBP) as a result of impaired 25(OH)D recirculation. In a study of type 1 diabetes patients, aged 14–40 years, with a disease duration of approximately 10 years, the excretion of uVDBP was reported to be exaggerated relative to the degree of albuminuria, and the frequency of vitamin D deficiency was found to be higher in albuminuric patients[3]. However, there have been no studies among pediatric patients with short disease durations and no albuminuria. In this study, we examined whether there would be an increased loss of uVDBP in pediatric type 1 diabetes patients without microalbuminuria and whether a correlation would exist between VDBP levels and vitamin D deficiency or circulating 25(OH)D levels. We also aimed to identify the risk factors that influenced low vitamin D levels in pediatric type 1 diabetes patients.

Materials and methods

1. Subjects

Patients with type 1 diabetes without microalbuminuria (n=42) and healthy control subjects (n=29), aged 9–14 years, were included in this hospital-based cross-sectional study in the capital region of Seoul (37°N), South Korea. The subjects were recruited from the pediatric endocrinology outpatient clinic at Seoul National University Children's Hospital. This study was conducted from January to March 2013 to exclude any seasonal variations. The exclusion criteria included a history of current or previous use of medications or medical conditions known to affect vitamin D metabolism or bone health, a history of malignancy or other autoimmune disease, restricted physical activity, short stature (<3rd percentile), obesity (body mass index [BMI] >95th percentile), or orthostatic proteinuria.

2. Measurements

The height, weight, and waist circumference were measured and pubertal status evaluated in all subjects. Height was measured with a Harpenden stadiometer (Holtain Ltd., Crymych, Wales, UK), and weight was measured with a digital scale. The BMI was calculated as the weight (kg) divided by the height squared (m^2). The minimal and umbilical waist circumferences were measured. The Z-scores of height and BMI were assigned according to the 2007 Korean National Growth Charts[9]. Pubertal onset was defined as breast budding in females and increased testicular volume (≥4 mL) in males. Bone age was assessed according to the Greulich and Pyle method.

The body fat percentage was measured using bioelectrical impedance analysis, Inbody 3.0 (Biospace Ltd., Seoul, Korea). Vitamin D deficiency was defined as a 25(OH)D level of <20 ng/mL[6]. The family history as well as previous medical and medication histories were evaluated using a questionnaire. A trained dietician collected information regarding vitamin D intake using a food-frequency questionnaire. The vitamin D content of foods were determined from the Rural Development Administration food composition tables[10], and the vitamin D contents of supplements were also identified. A questionnaire was used to assess the amount and intensity of physical activity including daylight outdoor hours and sedentary hours. The subjects' levels of physical activity were investigated during a normal week. The definition of regular physical activity was based on the physical activity guidelines of the United States Department of Health and Human Services[11]. Subjects with physical activity (moderate or vigorous-intensity physical activity) of at least 60 min/day for 7 days per week were considered to have participated in regular physical activity.

Serum levels of calcium, phosphorus, alkaline phosphatase, 25(OH)D, $1,25(OH)_2D$ were measured. Whole blood was obtained in ethylenediaminetetraacetic acid (EDTA) and sodium hepatin cell preparation tubes for quantification of intact parathyroid hormone (PTH) and serum VDBP (sVDBP). EDTA tubes were centrifuged at $1,000 \times g$ for 15 minutes within 30 minutes of collection and stored at -70℃ for analysis. A random urine sample was used to measure the microalbumin and creatinine (Cr). Random urine samples for uVDBP were stored at -70℃. The serum intact PTH level was measured using the standard ELSA-PTH immunoradiometric assay (CIS Bio International, Sorgues, France) with an interassay coefficient of variation (CV) of 4.6% and an intra-assay CV of 4.3%. The serum 25(OH)D level was measured using an ^{125}I-labeled radioimmunoassay (DiaSorin, Stillwater, MN, USA) with an interassay CV of 10.8% and an intra-assay CV of 9.4%. The serum 1,25(OH)2D level was measured using an ^{125}I-labeled radioimmunoassay (DiaSorin) with an interassay CV of 8.6% and an intra-assay CV of 13.8%. The VDBP levels were measured in duplicate by commercial enzyme-linked immunosorbent assay (Quantikine ELISA no. DVDBP0; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions with a normal range of serum 56 to 473 μg/mL and urine 0–250 ng/mL. The intra- and interassay coefficients of variation for this assay were 5.9% and 6.2%, respectively. The urine microalbumin and uVDBP values were normalized to the urine Cr concentrations and expressed as a microalbumin to Cr ratio (mACR, mg/g) and as a uVDBP to Cr ratio (uVDBPCR, ng/g), respectively, to control for variable urine concentrations. Normoalbuminuria was defined as mACR≤30 mg/g.

3. Statistical analysis

Statistical analyses were performed with IBM SPSS ver. 18.0

(IBM Co., Armonk, NY, USA). All continuous variables were described as mean±standard deviation. The variables were tested for normal distributions. Vitamin D intake, daylight outdoor hours (hr/wk), sVDBP, uVDBPCR, and mACR had skewed distributions and were therefore log-transformed for the analysis. Student t-test was used to compare the mean values, and the chi-square test was used to compare categorical variables between 2 groups with normally distributed data. Pearson correlation coefficients were assessed as the measure of correlation between variables of interest. A univariate linear regression analysis was performed to identify the risk factors of a low 25(OH)D level. A multivariate regression analysis was subsequently performed and included all significant independent variables from the univariate analysis and the previously known covariates (sex, body fat percentage, presence of type 1 diabetes, and the uVDBPCR). For all analyses, a P-value <0.05 was considered significant.

4. Ethics Statement

The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (approval number: H-1211- 083-442). Written informed consent was obtained from a parent or guardian as well as the subjects.

Results

1. Baseline participant characteristics

The patients with type 1 diabetes were older than those in the control group (12.2±1.8 years for patients with type 1 diabetes vs. 11.0±1.3 years for controls, P=0.002). There were no differences with regard to sex, pubertal status, height Z-score, BMI Z-score, and body fat percentage between the 2 groups. In the patients with type 1 diabetes, the disease duration was 6.4±3.0 years (range, 1.1–12.0 years), and the hemoglobin A1c level was 8.6%±1.2%. There were no differences with regard to vitamin D intake, daylight outdoor hours, regular physical activity and frequency of sunscreen use between the groups. Patients with type 1 diabetes had more sedentary hours (P=0.004) than the controls (Table 1).

Table 1. Demographic characteristics of the study subjects

Characteristic	Control (n=29)	Type 1 diabetes (n=42)	P-value
Male sex	13 (44.8)	12 (28.6)	0.159
Chronological age (yr)	11.0±1.3	12.2±1.8	0.002
Bone age (yr)	11.7±1.7	12.8±2.1	0.010
Puberty	20 (69.0)	35 (83.3)	0.154
Height Z-score	0.03±0.9	0.23±1.1	0.435
BMI Z-score	0.06±0.8	-0.12±0.8	0.377
Body fat (%)	23.6±6.4	23.1±7.0	0.815
Total vitamin D intake (μg/day)[*]	8.1±6.9	9.9±8.4	0.429
Sunscreen use	3 (10.3)	9 (21.4)	0.221
Daylight outdoor hours (hr/wk)[*]	11.2±8.1	16.3±14.3	0.232
Regular physical activity	3 (10.3)	6 (14.3)	0.624
Sedentary hours (hr/wk)[†]	15.0±10.4	22.7±10.8	0.004

Values are presented as number (%) or mean±standard deviation.
[*]Natural log transformed values were used for the analysis. [†]Hours of watching television or a computer.

Table 2. Comparison of metabolic characteristics between the control and type 1 diabetes groups

Variable	Control (n=29)	Type 1 diabetes (n=42)	P-value
25(OH)D deficiency	13 (44.8)	24 (57.1)	0.307
25(OH)D (ng/mL)	21.3±6.0	20.0±6.4	0.426
Serum VDBP (μg/mL)[*]	175.0±84.6	200.8±75.2	0.108
Urine VDBP (ng/mL)[*]	34.0±31.0	48.2±35.9	0.127
Urine VDBP/Cr (ng/mg)[*]	55.4±49.3	72.6±53.3	0.305
Urine microalbumin/Cr (mg/g)[*]	11.1±7.5	14.0±6.2	0.012
Total calcium (mg/dL)	9.6±0.4	9.6±0.3	0.920
Phosphorus (mg/dL)	4.9±0.5	5.0±0.8	0.545
Alkaline phosphatase (IU/L)	224.7±73.4	249.2±107.5	0.257
Parathyroid hormone (pg/mL)	22.7±12.7	22.3±11.4	0.902

Values are presented as number (%) or mean±standard deviation.
25(OH)D, 25-hydroxyvitamin D; VDBP, vitamin D-binding protein; Cr, creatinine.
[*]Natural log transformed values were used for the analysis.

2. Biochemical parameters of the participants

There was no significant difference in the frequency of vitamin D deficiency during the winter season (44.8% for controls vs. 57.1% for patients with type 1 diabetes). The serum 25(OH)D levels did not show any differences between the 2 groups. The sVDBP and uVDBPCR did not show any difference between the 2 groups. The urine mACR was significantly higher in the patients with type 1 diabetes than in the control group (P=0.012). There were no differences in serum levels of calcium, phosphorus, alkaline phosphatase and PTH between the groups (Table 2).

3. Association between VDBP and serum 25(OH)D levels or vitamin D deficiency

The sVDBP and uVDBPCR levels did not differ between the vitamin D deficiency and nondeficiency groups (Table 3). The uVDBPCR positively correlated with mACR in both groups (control: r=0.400, P=0.031; type 1 diabetes: r=0.614, P<0.001). The 25(OH)D level did not correlate with the sVDBP or uVDBPCR in both groups.

4. Factors associated with low serum 25(OH)D level

We performed univariate analyses to determine the risk factors of a low serum 25(OH)D level, using the serum 25(OH)D levels as the outcome variable. Low total vitamin D intake (P=0.005), older age (P=0.039) and reduced daylight outdoor hours (P=0.015) were significantly associated with a low vitamin

D status (Table 4). A multivariate regression analysis including the known risk factors for vitamin D deficiency such as age, sex, body fat percentage, vitamin D intake, daylight outdoor hours, presence of type 1 diabetes, and the uVDBPCR[3,12,13] revealed that both the presence of type 1 diabetes and uVDBPCR were not risk factors for vitamin D deficiency. The factors that affected 25(OH)D levels were daylight outdoor hours (β=2.948, P=0.003) and total vitamin D intake (β=2.865, P=0.003) (Table 4).

Discussion

The prevalence of vitamin D deficiency in pediatric patients with type 1 diabetes without microalbuminuria during the winter season (57.1%) was not different from a normal control group. The sVDBP and uVDBPCR did not show any difference between the 2 groups. The serum 25(OH)D level did not correlate with uVDBPCR. In pediatric type 1 diabetes patients with normoalbuminuria, the factors that affected serum 25(OH)D levels during the winter period were daylight outdoor hours and the vitamin D intake. The uVDBPCR did not correlate with the serum 25(OH)D levels.

In our study, uVDBPCR showed positive correlation with mACR as in the previous studies[3,14]. However, the uVDBPCR did not show any difference between the 2 groups in the setting of normoalbuminuria. VDBP is synthesized in hepatocytes and has a strong structural homology with albumin and a similar molecular weight (69 kDa for albumin vs. 58 kDa for VDBP)[15,16]. Furthermore, both albumin and VDBP uptake in the proximal tubule are principally controlled by megalin and

Table 3. Comparison of vitamin D and related analytes according to vitamin D status

Variable	Control			Type 1 diabetes		
	VDD (n=13)	ND (n=16)	P-value	VDD (n=24)	ND (n=18)	P-value
25(OH)D (ng/mL)	15.8±2.6	25.7±3.9	<0.001	15.4±3.4	26.2±3.5	<0.001
Serum VDBP (µg/mL)*	172.3±78.1	177.2±92.0	0.998	205.6±71.3	194.2±81.7	0.478
Urine VDBP (µg/mL)*	32.1±20.4	35.5±38.2	0.997	54.7±40.8	39.7±26.7	0.251
Urine VDBP/Cr (ng/mg)*	53.3±34.0	57.1±60.1	0.947	80.6±58.8	62.0±44.3	0.333

Values are presented as mean±standard deviation.
VDD, vitamin D deficiency; ND, nondeficiency; 25(OH)D, 25-hydroxyvitamin D; VDBP, vitamin D-binding protein; Cr, creatinine.
*Natural log transformed values were used for the analysis.

Table 4. Results of unadjusted and multivariate-adjusted linear regression analyses with 25-hydroxyvitamin D concentrations as the dependent variable in study subjects (n=71)

Variable	Unadjusted			Multivariate-adjusted		
	β	SE	P-value	β	SE	P-value
Age	-0.890	0.423	0.039	-0.655	0.460	0.160
Female sex	-1.894	1.543	0.224	-0.970	1.593	0.545
Body fat	-0.146	0.110	0.191	-0.093	0.117	0.430
Total vitamin D intake*	2.721	0.940	0.005	2.865	0.923	0.003
Daylight outdoor hours*	2.435	0.974	0.015	2.948	0.953	0.003
Type 1 diabetes	-1.209	1.508	0.426	-1.267	1.586	0.428
Urine VDBP/Cr*	-0.207	0.974	0.832	-0.427	0.909	0.641

SE, standard error; VDBP, vitamin D-binding protein; Cr, creatinine.
*Natural log transformed values were used for the analysis.

its coreceptor cubilin[17]. Therefore, it seems that the VDBP and albumin go through similar process in the kidney. Regarding sVDBP, there was a report of decreased sVDBP levels in diabetic rats[18], and patients with type 1 diabetes have been found to have lower sVDBP levels when compared to controls[19]. However, according to a recent study, the sVDBP levels did not differ between patients with type 1 diabetes and the control group[3] as in our study.

There are not many studies which investigated the relationship between uVDBP and 25(OH)D levels[3,20]. In a study conducted in diabetic DBA/2 J nephropathy-prone mice, urinary 25(OH)D excretion increased along with increasing uVDBP excretion[21]. In albuminuric patients with type 1 diabetes, urinary megalin and cubilin excretion was increased, along with uVDBP[22]. Thrailkill et al.[3] reported that uVDBP excretion in type 1 diabetes patients aged 14–40 years was higher than that in the control group, and was further increased in patients with albuminuria. Furthermore, vitamin D deficiency and insufficiency were more common in patients with type 1 diabetes with albuminuria, coincident with the increase in uVDBP excretion. However, uVDBPCR did not associate with the serum 25(OH)D levels as in our study. This may be attributed to the small number of patients in the albuminuria group (9.4%) and the even smaller number of macroalbuminuria patients (1.18%) analyzed. Regarding the association between sVDBP and 25(OH)D level, most studies reported of no association[14,19,23,24] except one study reporting a positive correlation[25]. The VDBP and 25(OH)D levels may vary independently because VDBP (5×10^{-6} mol/L) circulates at a concentration approximately 100 fold higher than that of its ligand 25(OH)D (5×10^{-8} mol/L)[26]. Only a severe and long-term loss of VDBP, as observed in nephrotic syndrome with continuous high-grade proteinuria, could lead to decreases in the 25(OH)D level[20].

Many studies found a higher prevalence of vitamin D deficiency in patients with type 1 diabetes when compared with the control group[27-29]. In this study, we did not find a significant difference in the prevalence of vitamin D deficiency between the 2 groups. Because the frequency of vitamin D deficiency is high in healthy Korean children during the winter period[30], the prevalence of vitamin D deficiency might not differ between the type 1 diabetes and control groups during this season.

The factors known to contribute to low vitamin D levels are high latitude, winter season, black race, old age, female sex, high BMI, low physical activity, and low vitamin D intake[12,13]. A study in type 1 diabetes reported season and the presence of type 1 diabetes as significant factors affecting vitamin D status[3]. In this study, we excluded obese subjects and performed an analysis during the winter period at the same latitude to minimize the influence of ultraviolet rays, which comprise a significant factor with respect to the vitamin D status. In our study, although the patients with type 1 diabetes were older than those in the control group, there was no significant difference in the prevalence of vitamin D deficiency between the groups during the winter period. The daylight outdoor hours as well as

vitamin D intake were the factor associated with the 25(OH)D level during the winter season. Stored vitamin D, generated via outdoor activities during summer and autumn period, may affect vitamin D status in the winter. The presence of type 1 diabetes per se did not impair 25(OH)D circulation.

The limitations of our study include the cross-sectional design and relatively small number of patients. However, this is the first study to analyze the association between the VDBP level (serum and urine) and the 25(OH)D level or vitamin D deficiency in pediatric patients with type 1 diabetes without microalbuminuria. We also included all of the known risk factors such as the body fat percentage, sex, vitamin D intake, and daylight outdoor hours in the analysis while controlling for the effects of the season, latitude, and obesity.

In conclusion, there was no correlation between serum 25(OH)D and uVDBP level in the setting of normoalbuminuria without considerable uVDBP excretion. The factors associated with the 25(OH)D levels during the winter period were the daylight outdoor hours and vitamin D intake, not the presence of type 1 diabetes or uVDBPCR. Therefore, sufficient vitamin D intake and daylight outdoor hours need to be emphasized in order to achieve improvements in vitamin D status.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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