ORIGINAL ARTICLE

HYPOVITAMINOSIS D IN CHILDREN WITH TYPE 1 DIABETES MELLITUS AND ITS INFLUENCE ON BIOCHEMICAL AND DENSITOMETRIC PARAMETERS

Jarmila Vojtková, Miriam Čiljaková, Lenka Vojarová, Katarína Janíková, Zuzana Michnová, Veronika Šagiová

Department of Pediatrics, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, Slovakia

Summary: The aim of the study was to establish the frequency of hypovitaminosis D in children with type 1 diabetes mellitus (T1D), its influence on biochemical and densitometric parameters and the relation to diabetic nephropathy. 58 children with T1D at the age 9–19 years were enrolled to the study. Vitamin D concentration less than 30 ng/ml was considered as insufficient. 37 children (63.79%) had vitamin D level under 30 ng/ml, from these 19 subjects (32.7%) had vitamin D level under 20 ng/ml and 2 subjects (3.44%) under 10 ng/ml. Children with vitamin D deficiency had significantly lower magnesium concentration and lower Z score of lumbar spine (−1.34 ± 1.24 vs. −0.30 ± 1.21, p = 0.01) compared to diabetics with sufficient vitamin D concentration. No significant difference was found in parameters calcium, phosphorus or glycosylated hemoglobin. Patients with diabetic nephropathy (n = 18) showed no significant difference in vitamin D, glycosylated hemoglobin or Z score of lumbar spine compared to the patients without nephropathy (n = 40). Subjects with nephropathy had significantly longer diabetes duration, significantly higher cholesterol and triacylglycerol concentration. In our cohort of patients nearly two thirds of children had insufficient vitamin D concentration what supports the need to monitor and eventually supplement vitamin D in T1D subjects.

Key words: *Vitamin D deficiency; Type 1 diabetes mellitus; Biochemical and densitometric parameters*

Introduction

Vitamin D represents a group of steroid hormonal precursors necessary for the synthesis of calcitriol – the hormon with important role in the calcium-phosphate metabolism and right bone formation (21). According to the recent information vitamin D also regulates the cell growth and differentiation, induces apoptosis of neoplastic cells, has immunomodulatory effect (2) and can prevent the cardiovascular diseases (13, 14, 33). Maternal vitamin D deficiency during pregnancy may lead to impaired fetal growth and bone development (4). In relation to carbohydrate metabolism, it can increase the pancreatic insulin secretion (23) and its deficit is associated with impaired glucose tolerance (25). Even 80% of vitamin D source is sunshine. Ultraviolet irradiation (UVB at wavelenghts 280–315 nm) splits provitamin 7-dehydrocholesterol in the skin into provitamin D_3 which can spontaneously change itself into an isomer, cholecalciferol, uptaken by the liver. About 20% of cholecalciferol comes from animal food sources (fish oil, liver, eggs, milk) absorbed through the intestine. In the liver, 25-hydroxycholecalciferol (25-OHD) arises by 25-hydroxylase. 25-OHD is the main form of vitamin D in the circulation, represents the majority of vitamin D reserves in the liver (22) and its huge part passes into the bile (enterohepatic circulation). The next important organ in vitamin D metabolism is kidney (16), where 1,25-dihydroxycholecalciferol [$1,25(OH)_2D$] or calcitriol arises by α1-hydroxylase. Normal concentration of serum vitamin D_3 is 30–100 ng/ml, concentration less than 30 ng/ml is considered as insufficient (Table 1).

Nowadays, many studies claim about increasing deficit of vitamin D in whole children and adolescent population (9, 12). The presence of type 1 diabetes mellitus (T1D) may even enhance this number (8). The prevalence of insufficient vitamin D level in subjects with diabetes mellitus varies between 50–90% according to different authors (19, 20) and may have relation to intestine microangiopathy with insufficient absorption or to nephropathy with inability of kidney to hydroxylate 25-OHD into $1,25(OH)_2D$

The aim of the study was to establish the prevalence of vitamin D deficiency in children with type 1 diabetes mellitus, to compare the biochemical and densitometric parameters between patients with insufficient and normal level of vitamin D and to find if diabetic nephropathy influenced these parameters.

Material and Methods

The study was approved by Ethical Commitee of Jessenius Faculty of Medicine in Martin, Slovakia and was performed according to the Declaration of Helsinki. Enrolled criteria were signed informed consent, age 9–19 years, diagnosed type 1 diabetes mellitus (T1D) based on criteria of

Tab. 1: Vitamin D concentrations

Normal value of vitamin D	30–100 ng/ml
Insufficiency of vitamin D	20–30 ng/ml
Deficit of vitamin D	10–20 ng/ml
Severe deficit of vitamin D	< 10 ng/ml

American Diabetes Association. Subjects were investigated during autumn time from September to November 2010. Immobile patients, patients treated by corticoids, by calcium, magnesium or vitamin D supplements and subjects with known diseases of calcium-phosphate metabolism (parathyreosis disorder, tetania, rachitis, other) were excluded from the study. Subjects with acute condition (respiratory infection, ketoacidosis) were enrolled to the study the soonest in 4 weeks after recovering.

Diabetes onset, diabetes duration and basic anthropometric parameters (weight, height, body mass index – BMI) were noticed in each patient. Microalbuminuria and so the presence of eventual diabetic nephropathy was investigated from 12-hour night urine sample while good glycemia compensation, normal blood pressure and without excessive physical activity. Patients had drawn 5 ml of venous blood, from which biochemical parameters – total calcium (Ca), ionized calcium (Ca^{2+}), phosphorus (P), magnesium (Mg), cholesterol, triacylglycerol and glycosylated hemoglobin (HbA1c) were established by standard biochemical methods and vitamin D_3 level by commercial kit Roche® Elecsys2010. All patients had examined densitometry by Hologic Discovery Bone Densitometer and Z score of lumbar spine and femur were established. The results were correlated considering the age, sex and "height age" (the age which reflects the child's height according to the 50th percentile of growth tables) (10).

The results were statistically processed by system MS Excel 2007. Numeric variables were parametrically distributed and were expressed as mean with standard deviation. To assess significant difference between subgroups Student's t-test was used and p less than 0.05 was considered statistically significant.

Results

58 children, 30 boys and 28 girls, with type 1 diabetes mellitus at the age 9–19 years (average 13.91 ± 2.95 years) were enrolled to the study. 21 children (36.21%) had normal concentration of vitamin D, however in 37 children (63.79%) insufficient vitamin D level (under 30 ng/ml) was found. From these patients 19 subjects (32.7%) had vitamin D level under 20 ng/ml and 2 subjects (3.44%) under 10 ng/ml (Figure 1).

Children with insufficient level of vitamin D had significantly lower magnesium concentration (0.79 ± 0.09 mmol/l vs. 0.84 ± 0.08, $p < 0.05$) and significantly lower total Z score of lumbar spine densitometry (−1.34 ± 1.24 vs. −0.30 ± 1.21, $p = 0.01$) compared to the children with normal vitamin D level (Figure 2). No significant difference was found in

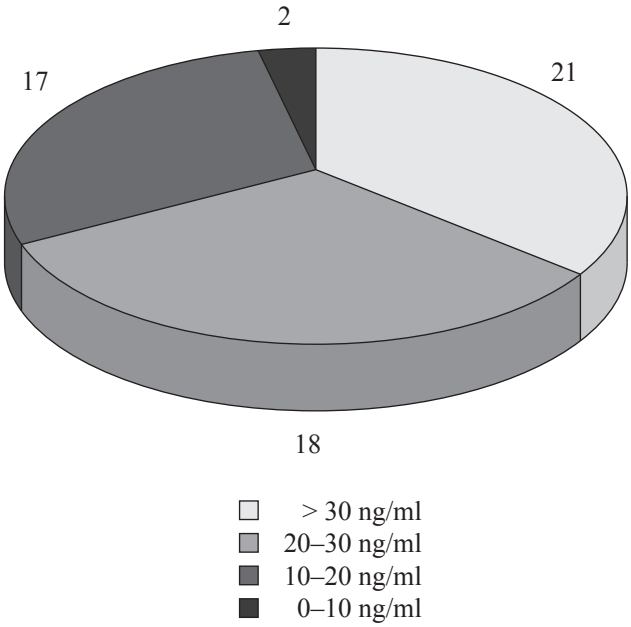

Fig. 1: Vitamin D level in children with diabetes mellitus

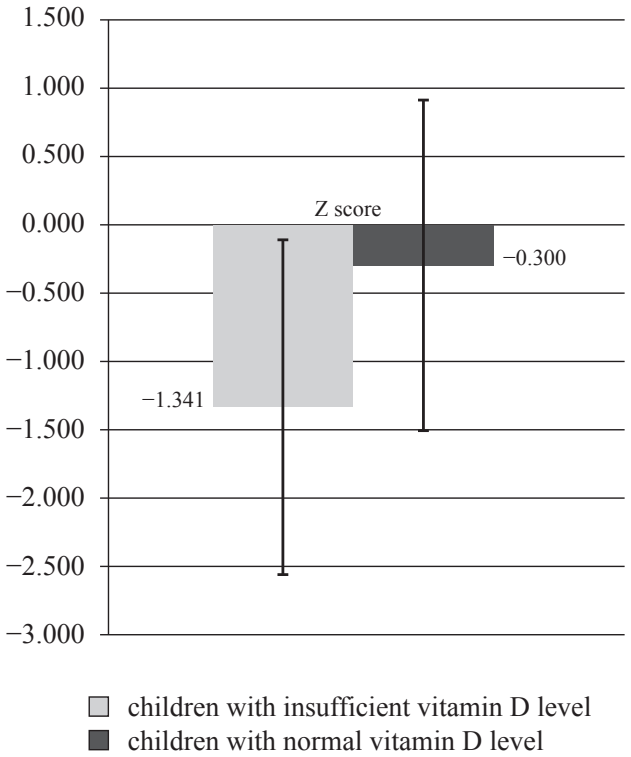

Fig. 2: Significantly lower Z score of lumbar spine densitometry in children with insufficient vitamin D level

Tab. 2: Differences in measured parameters between children with insufficient and sufficient level of vitamin D

	Children with insufficient level of vitamin D (n = 37)	Children with sufficient level of vitamin D (n = 21)	p
Diabetes duration (years)	5.46 ± 4.07	5.30 ± 4.00	> 0.05
Diabetes onset (years)	7.85 ± 4.09	9.55 ± 3.71	> 0.05
Ca (mmol/l)	2.43 ± 0.12	2.47 ± 0.12	> 0.05
Ca^{2+} (mmol/l)	1.20 ± 0.11	1.22 ± 0.09	> 0.05
P (mmol/l)	1.39 ± 0.24	1.36 ± 0.25	> 0.05
Mg (mmol/l)	0.79 ± 0.09	0.84 ± 0.08	**< 0.05**
HbA1c (%)	10.62 ± 2.84	10.49 ± 1.92	> 0.05
Cholesterol (mmol/l)	4.64 ± 1.08	4.59 ± 0.99	> 0.05
Triacylglycerol (mmol/l)	1.25 ± 0.77	1.36 ± 1.02	> 0.05
BMI (kg/m^2)	19.32 ± 3.52	20.23 ± 3.70	> 0.05
Microalbuminury (mg/l)	8.09 ± 10.47	8.57 ± 16.11	> 0.05
Z score lumbar spine	−1.34 ± 1.24	−0.30 ± 1.21	**= 0.01**
Z score femur	−1.04 ± 0.96	−0.50 ± 0.70	= 0.06

parameters like diabetes duration, total or ionized calcium, phosphorus, glycosylated hemoglobin, cholesterol and triacylglycerol between these two groups (Table 2).

Based on microalbuminury, incipient nephropathy was established in 18 diabetic patients. No significant difference was found in vitamin D concentration, calcium, phosphorus, magnesium, glycosylated haemoglobin or Z score densitometry between diabetic patients with or without nephropathy. Patients with nephropathy had significantly longer diabetes duration (7.77 ± 3.58 years vs. 4.40 ± 3.79, p = 0.001), significantly higher cholesterol (4.96 ± 0.77 mmol/l vs. 4.48 ± 1.12, p < 0.05), triacylglycerol level (1.70 ± 0.97 mmol/l vs. 1.11 ± 0.75, p < 0.05) and body mass index (20.97 ± 3.71 kg/m^2 vs. 19.05 ± 3.39, p < 0.05) compared to the patients without nephropathy (Table 3).

Discussion

Association of diabetes mellitus with disorder in bone metabolism and changes in vitamin D level is not completely understood. At least three hypotheses exist about the relation of these clinical units. It could represent two separate problems, probably as a consequence of improper eating habits and insufficient sunbathing (26). Another possibility is that diabetes mellitus is a cause for vitamin D deficiency (6, 20) as a consequence of intestine microangiopathy with insufficient absorption or kidney inability to hydroxylate 25-OHD into $1,25(OH)_2D$ (29). The third hypothesis is that vitamin D deficiency is one of the factors predisposing to diabetes mellitus (24, 32) what is supported by new information about immunomodulatory impact of vitamin D (1). Insufficient level could predispose to origin of allergy (17, 18) or autoimmune diseases as type 1 diabetes mellitus.

Nowadays, a lot of studies claim about decreased level of vitamin D in diabetic patients with prevalence 50–90% according to the different authors (19, 20). Deficit of vitamin D was significantly more frequent (90.6%) and vitamin D level was significantly lower in diabetic children under 16 years compared to the healthy children (85.3%) with matched age (3). Sufficient level of vitamin D was established in less than 25% of 128 patients with T1D (30). Subjects with vitamin D deficit had significantly longer diabetes duration and were significantly older (at the age 12–18 years). Similarly, significantly lower vitamin D concentration was found in 26 diabetic patients compared to 15 non-diabetic patients (34). In our group of patients, vitamin D deficiency was found in 63.79% of patients with T1D and subjects with insufficient level of vitamin D had significantly lower bone density of lumbar spine compared to the patients with normal value of vitamin D. No significant differences were found in serum phosphorus, total and ionized calcium level between observed groups of patients, what may reffer to adequate compensation mechanisms maintaining the normal concentration of these ions.

Magnesium is essential for the formation of $1,25(OH)_2$ vitamin D_3, adequate function of the parathyroid glands and sensitivity of target tissues to parathyroid hormone and vitamin D (35). Magnesium deficit is associated with hypoparathyroidism, low production of active vitamin D metabolites and resistance to parathormone and vitamin D (7). Several studies have shown that magnesium supplementation may improve bone mineral density (31). Magnesium is also critical for insulin sensitivity and its release. In subjects with type 2 diabetes, hypomagnesemia may worsen insulin resistance (28). Hypomagnesemia is usually seen in subjects with diabetes mellitus especially in those with inadequate compensation. Hyperglycemia is associated with magnesiuria and calciuria as the kidney is not able to reabsorb these ions (15). Absorption of magnesium through small intestine is primarily via passive diffusion independent of vitamin D, however vitamin D supplementation increases magnesium absorption (11). Calcium and magnesium absorption

Tab. 3: Differences in measured parameters between children with and without diabetic nephropathy

	DM with incipient nephropathy (n = 18)	DM without nephropathy (n = 40)	p
Diabetes duration (years)	7.77 ± 3.58	4.40 ± 3.79	**= 0.001**
Diabetes onset (years)	7.71 ± 3.04	8.75 ± 4.35	> 0.05
Ca (mmol/l)	2.43 ± 0.08	2.46 ± 0.13	> 0.05
Ca^{2+} (mmol/l)	1.26 ± 0.09	1.19 ± 0.09	> 0.05
P (mmol/l)	1.39 ± 0.27	1.38 ± 0.24	> 0.05
Mg (mmol/l)	0.81 ± 0.09	0.82 ± 0.09	> 0.05
HbA1c (%)	10.89 ± 1.76	10.43 ± 2.82	> 0.05
Cholesterol (mmol/l)	4.96 ± 0.77	4.48 ± 1.12	**< 0.05**
Triacylglycerol (mmol/l)	1.70 ± 0.97	1.11 ±0.75	**< 0.05**
BMI (kg/m^2)	20.97 ± 3.71	19.05 ± 3.39	**< 0.05**
Vitamin D (ng/ml)	29.52 ± 10.03	24.87 ± 11.74	> 0.05
Z score lumbar spine	−1.06 ± 1.18	−0.89 ± 1.37	> 0.05
Z score femur	−1.27 ± 0.83	−0.69 ± 0.89	> 0.05

is competitive – high calcium intake may decrease magnesium absorption and low magnesium intake may increase calcium absorption. In our study, subjects with vitamin D deficiency had significantly lower magnesium concentration what may be explained by few hypotheses. First, hypomagnesemia may be predisposing factor for vitamin D deficiency and impaired bone density. Second, vitamin D deficiency may cause hypomagnesemia through decrease of intestine absorption. Third, magnesium concentration is related to diabetes mellitus and hyperglycemia causing hypermagnesiuria, however in our study no difference of diabetes compensation was found between subjects with lower and normal magnesium concentration.

T1D negatively influences the bone density and increases the risk of bone fractures. One of the consequences of diabetes as chronic disease is osteopenia caused by decreased osteoblastic acitivity while normal resorption ability. Osteopenia is related to deficit of insulin, which has anabolic function (27). Osteopenia is also seen in patients with insufficient diabetes compensation. Other possible interpretation of decreased bone density in diabetic patients is metabolic changes, for example advanced glycation end products in bone collagen, hypercalciuria associated with glycosuria and magnesiuria, inflammatory cytokines or diabetic microangiopathy with reduced blood supply of bone tissue (30). Possible cause of vitamin D defficiency in diabetic patients is also chronic diabetic complication – diabetic nephropathy. Danish authors claim negative correlation between decreased bone density and microalbuminury in adult patients with T1D (5), therefore diabetic patients with microalbuminury had significantly lower bone density. Diabetic osteopenia may be related to diabetic nephropathy as kidney ability to hydroxylate the vitamin D can be afflicted with consequence of insufficient formation of vitamin D_3. In our study, we did not claim the influence of nephropathy on vitamin D deficiency what could be caused by incipient phase of nephropathy in young diabetic population.

Conclusion

In our study, nearly two thirds of children with type 1 diabetes mellitus had insufficient level of vitamin D with negatively influence on densitometric parameters. Diabetic patients should be monitored in vitamin D concentration and should be recommended to modificate their life-style with including the animal sources of vitamin D to their nutrition and to sunbath face and hands ten to twenty minutes daily according to the colour of the skin. If necessary, patients should be treated by vitamin D and magnesium supplement according to individual needs or during the winter and spring period.

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Corresponding author:

MUDr. Jarmila Vojtková, PhD., Department of Pediatrics, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, Kollárova 2, 036 01 Martin, Slovakia; e-mail: jarmilavojtkova@gmail.com