Vitamin D Deficiency is Comparatively More Prevalent in Female Children with Type 1 Diabetes in a High Vitamin D Deficiency Risk Country

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Abstract

Background: Vitamin D plays a very important role in maintaining a healthy skeleton. An individual with a deficiency of vitamin D is at a higher bone fracture risk. Type 1 diabetic adolescents show a lower peak bone mass in comparison with healthy peers, which may increase the risk of bone fracture at a later age. Thus, maintenance of a sufficient vitamin D level through optimal supply may be significantly important for healthy bones in children with type 1 diabetes.

Methods: This study comprised 100 children with type-1 diabetes and 100 non-diabetics. Serum 25-hydroxy-vitamin D, PTH, total serum calcium, phosphate, and alkaline phosphatase, were measured. Age, gender, and duration of type 1 diabetes were accounted. Body mass index was also calculated. The data obtained were analyzed by SPSS v.19 program according to the age, sex and vitamin D deficiency level.

Results: Out of 100 children 84 (84%) were vitamin D deficient (compared to control; 58%), defined as a 25-hydroxy-vitamin-D level below 50 nmol/L. The deficiency was found directly proportional to the age. Female children showed higher prevalence of vitamin D deficiency compared to males (59%). Despite the high prevalence of vitamin D deficiency, we found a lower prevalence of secondary hyperparathyroidism in children and adolescent patients.

Conclusions: Prevalence of vitamin D deficiency in diabetic children and adolescents is high. Females are significantly associated with the vitamin D deficiency. Therefore, screening for vitamin D deficiency in children and particularly in girls is recommended in the studied population.

Introduction

Vitamin D is a fat-soluble vitamin. It has the important function role in bone metabolism and possibly has some anti-inflammatory and immune-modulating properties. Vitamin D is a precursor hormone and the building block of a steroid hormone calcitriol. It is usually found in two forms. One is vitamin D2, present in plants and some fish and the other is cholecalciferol, or vitamin D3, and is synthesized in the skin by sunlight. Vitamin D requirements can be fulfilled either by ingesting vitamin D or by exposure to sun for a sufficient time to produce required amounts. Vitamin D has an important role in calcium absorption in the small intestine. Along with parathyroid hormone, it maintains calcium homeostasis in the blood stream by mediating skeletal mineralization of the same [1]. Hence, vitamin D has a major impact on bone health. Severe vitamin D deficiency may cause rickets in infants [2] and osteomalacia in adults [3]. A higher bone fracture risk in elderly people is accompanied with low serum vitamin D levels. [4]. In addition, vitamin D deficiency is common in children with type 1 diabetes mellitus (T1DM) [5].

The most accurate way to measure vitamin D content in the human body is the 25-hydroxy vitamin D (25D) assessment in blood serum [6]. Although, the American Academy of Pediatrics has recommended the serum level of 25D below 27.5 nmol/L in children as vitamin D deficiency [7]. As a matter of fact, rickets in infants and children has been reported at 25D levels below 37.5 nMol/L [8, 9]. However, there is no consensus on the 25D levels that can be regarded as sufficient in children, to date. Hence, in the present study a cut off value of 25D, <50nMol/L was opted as deficiency [6].
Diabetes mellitus has an adverse effect on the skeleton and seemingly increases the risk of osteoporosis as well as fragility fractures. The exact mechanism of lowering the bone strength is not fully understood but may include impaired accrual of peak bone mass and diabetic complications, such as nephropathy. T1DM is more damaging than type 2 diabetes mellitus (T2DM) for strength of bone [10]. A lower bone mass in children with T1DM compared to healthy ones has been reported in many clinical studies [11].

However, there is a lack of adequate studies on association of vitamin D deficiency in diabetic children from the Middle East region. A clinical study on a Qatari population showed that the severe vitamin D deficiency was considerably prevalent in children with T1DM compared to healthy children [12]. Due to the high rate of incidence of vitamin D inadequacy in children and adolescents with T1DM, a routine screening for vitamin D deficiency, optimal vitamin D supply and close follow-up are being recommended [10, 13].

The objective of the present study was to assess the prevalence of vitamin D deficiency in children and adolescent patients with T1DM and to determine the factors which may possibly influence serum vitamin D levels. For example, age, serum calcium, serum phosphate, serum alkaline phosphatase, body mass index and duration of diabetes. In addition to that, intact parathyroid hormone (iPTH) concentration was also determined to assess secondary hyperparathyroidism in patients with vitamin D deficiency. A prospective, cross-sectional study was performed in children and adolescents with T1DM examined during January to December 2011 at Security Forces Hospital, Riyadh, Saudi Arabia.

Subjects and Methods

Study Subjects

One hundred Saudi children (59 females and 41 males) with T1DM (of more than 5 months duration) and 100 non-diabetic children (52 females and 48 males) were selected. The age of children included in this study was in the range of 2-17 years. Children on vitamin D, calcium supplementation, or with diabetic complications, such as diabetic nephropathy and those suffering from chronic illnesses, such as gastrointestinal disorders, malabsorption and celiac disease were excluded from study. The study was approved by the University Review Board at King Saud University and the Ethical Committee of the concerned hospital. A written informed consent was taken from the patient's guardians. A questionnaire for evaluating the daily consumption of dairy products and calcium-rich mineral water and exposure to sun was used. General physical examinations of patients were measured using standard methods [14]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m2) [15].

Laboratory Findings

Quantitative biochemical assays for 25D, iPTH and bone profile (calcium, magnesium, phosphorus, and alkaline phosphatase) were performed. Serum iPTH was determined using an electrochemiluminescent immune assay (Roche Modular E170, Roche Diagnostics Corp., Indianapolis, USA). Serum 25D levels was determined by using the high-performance liquid chromatography method. Due to the lack of consensus on the titer of 25D as sufficient for healthy individuals we considered a mild deficiency as concentration of 25 D <50 nMol/L, moderate deficiency <25 nMol/L and severe deficiency <12.5 nMol/L. The HbA1c was measured using high-performance liquid chromatography (reference range: 4-6%).

Statistical Analysis

Results are expressed as mean ± standard error of mean (SEM). All the statistical analysis was performed using the SPSS 19 program (PSS Inc. Chicago, IL, USA). Student's t-test was performed to compare the means between male and female groups of control and patients. For detailed analysis patients and controls were grouped according to the deficiency of vitamin D as severe deficiency, moderate deficiency and mild deficiency. Differences of means among the groups were analyzed by one-way ANOVA. *X*2- test was performed to find out the differences in proportions of control and patients in

the three groups. Differences of means were considered significant at P<0.05. All the results were tabulated into contingency tables.

Results

In the present study 100 children and adolescents with T1DM and 100 non-diabetic children were assessed for the clinical and laboratory findings. None of the participants had any history of low intensity trauma bone fracture or any bone deformities. Forty one (41%) patients were males and fifty nine (59%) were females whereas forty eight (48%) controls were males and fifty two (52%) were female. Most of the patient children were white or brownish white (92%) and the remaining were black (8%). Among the controls 89% were white and 11% were black. The mean (±SEM) of assayed biochemical and physical parameters of the whole group is shown in Table 1 and those of sex wise two groups in Table 2. The mean of 25D in patients was 36.78 ± 1.43 nMol/L which was significantly lower (p<0.001) than that of controls 46.05 ± 1.40 nMol/L. Serum phosphate was also significantly lower in patients in comparison with controls (p<0.05). However, serum alkaline phosphatase of patients was significantly higher than that of controls (p<0.001)(Table-1).

PTH, calcium and phosphate levels were almost similar in patients and controls of females (p=0.59, 0.21, and 0.15 respectively). However calcium and phosphate levels were significantly (p<0.05 and 0.01) lower (2.28 ± 0.02 mMol/L and 1.56 ± 0.04 mMol/L respectively) in male patients compared to male controls (2.34 ± 0.15 mMol/L and 1.63 ± 0.02 mMol/L respectively). 24D in male and female patients were 39.58 ± 2.19 nMol/L and 34.83 ± 1.98 nMol/L respectively and significantly lower than that of male and female controls (p<0.001 and =0.05 respectively). Alkaline phosphatase was significantly higher in both male and female patients compared to respective controls (p<0.01 and 0.05 respectively) and were lower in male patients than that of male controls (Table 2).

Out of 100 patients 84 were vitamin D deficient whereas 58 controls were vitamin D deficient out of 100 (Table 3). A comparison of different clinical and biochemical parameters among severe, moderate and mild vitamin deficient patients and controls are tabulated in Table 3 (next page). Although, no statistically significant differences were found among the patients and controls of three vitamin deficient groups for their age, serum phosphate level and body mass index (p=0.47, 0.66 and 0.52 respectively). However, the age of severely, moderately and mildly vitamin deficient patients were 11.75 ± 0.85, 10.0 ± 0.53 and 9.77 ± 0.42 respectively. Serum calcium level was lowest (2.17 ± 0.04, p<0.05) in severely vitamin deficient patients compared to respective controls as well as patients and controls of the other two groups. Serum PTH was 75.0 ± 26.65 and 80.14 ± 28.84 mMol/L for severely vitamin D deficient patients and moderately deficient controls which were not greatly different. Interestingly, longest duration of T1DM was found in severely deficient patients (52.5 ± 22.89 months) and followed by moderately and mildly vitamin deficient patients (p<0.05).

Discussion

In this study, we found that children with T1DM have a high prevalence of vitamin D deficiency (84 patients compared to 58 in controls). Vitamin D levels showed 25D deficiency directly proportional to the age (Table 4 - page 34). Earlier, Svoren et al (2009) produced a similar report in which vitamin D deficiency was more prevalent among older children [13]. Contrarily, in a Swedish population serum 25D levels were above 50 nMol/L in teenagers with T1DM [16]. Female patients had a lower 25D level (34.83 ± 1.98 nMol/L) than males (39.58 ± 2.19 nMol/L) (Table 2 - opposite). Furthermore, moderate and mild vitamin D deficient children and adolescents with T1DM have a low prevalence of secondary hyperparathyroidism compared to severe vitamin D deficiency (Table 4). Similarly, level of serum calcium was significantly lower in severely deficient patients than

	Mean ± SEM		*p*-value
Characteristics	Patients	Controls	
Age (Years)	9.53 ± 0.32	8.36 ± 0.2	<0.01
25-hydroxy vitamin D (nM/L)	36.78 ± 1.43	46.05 ± 1.40	<0.001
Parathyroid Hormone (pg/mL)	47.82 ± 2.16	47.26 ± 4.26	0.90
Calcium (mM/L)	2.32 ± 0.01	2.33 ± 0.01	0.59
Phosphate (mM/L)	1.54 ± 0.02	1.6 ± 0.02	<0.05
Alkaline Phosphatase (Units/L)	274.84 ± 7.83	233.76 ± 6.22	<0.001
Body Mass Index	17.64 ± 0.36	17.88 ± 0.29	0.62
Duration of Type 1 diabetes (Months)	38.24 ± 3.26	NA	NA

Table 1: Comparison of means (± SEM) between patients and control's biochemical and physical characteristics

Characteristics	Male		p-value	Female		p-value
	Patients	Controls		Patients	Controls	
Age	8.98 ±0.42	8.04 ± 0.31	<0.05	9.9 ± 0.47	8.65 ± 0.26	<0.02
25-hydroxy vitamin D (nM/L)	39.58 ± 2.19	51.04 ± 1.62	<0.001	34.83 ± 1.98	41.44 ± 2.06	0.05
Parathyroid Hormone (pg/mL)	43.61 ± 3.39	39.2 ± 2.34	0.53	50.74 ± 2.80	54.69 ± 7.81	0.59
Calcium (mM/L)	2.28 ± 0.02	2.34 ± 0.15	<0.05	2.35 ± 0.01	2.32 ± 0.01	0.21
Phosphate (mM/L)	1.56 ± 0.04	1.63 ± 0.02	<0.05	1.52 ± 0.03	1.58 ± 0.02	0.15
Alkaline Phosphatase (Units/L)	276.48 ±11.85	226.89 ±9.09	<0.01	273.69 ±10.7	240.09 ±8.52	<0.05
Body Mass Index	17.48 ± 0.52	17.4 ± 0.39	0.7	17.74 ± 0.49	18.32 ± 0.43	0.22
Duration of Type 1 diabetes (Months)	29.7 ± 3.82	NA		44.13 ± 4.72	NA	

Table 2: Mean of biochemical and physical characteristics of patients compared with controls according to their sex.

those of moderate and mild deficiency patients (Table 4; Figures 1 & 2 - following pages).

Most importantly, we found that the prevalence of vitamin D deficiency in the studied population is very much higher than other studies in Australia, USA and Sweden etc. In the studied Saudi population, 84% of the children with T1DM were found to be vitamin deficient. This percentage of prevalence is much higher than that of 60.5% in a Swiss population [5], 43% in an Australian population [17], 25% in an Italian population [18] and 15% in a North American population [13]. However, a study from Qatar reported a prevalence of vitamin D deficiency in 90.6% children with T1DM [12]. This variability may be due to differences in food behavior, exposure to sun, geographical conditions, skin complexion, and/or genetic constitutions.

Prevalence of secondary hyperparathyroidism was high in the severely vitamin D deficient group. However, secondary hyperparathyroidism was not observed in very low, in moderate, or mild vitamin D deficient patients. In contrast, PTH levels never fell below 40 pg/ml in any of the sub groups of studied subjects. That is higher than 10 pg/ml expected for the continuous secretion of PTH [19]. In adults, PTH levels are expected to rise steeply above 40 pg/ml at 25D levels below 50 nmol/L and above 50 pg/ml at 25 D levels below 25 nmol/L [20]. This relationship seems to be less pronounced in healthy adolescents [21]. No expected rise in PTH level in diabetic children above 7 years in our study can be explained by the following facts; (i) PTH-vitamin D axis has a blunted response in diabetic patients [22]. In one study a blunted response of PTH was associated with low magnesium levels and corrected after magnesium repletion [23] and (ii) hypocalcaemia was very mild, so it would not been sufficient to induce a rise in PTH in patients [19].

Major limitations of our study include inaccuracy of calcium rich diet (milk, fish etc.) measurement, and the exact duration of sun exposure and seasonal change in serum vitamin D level fluctuation. In conclusion, our study reported that vitamin D deficiency is directly proportional to the duration of diabetes and highly prevalent in female patients in the studied population. Furthermore, a low prevalence of secondary hyperparathyroidism was found in vitamin D deficient children. The present study recommends the screening of vitamin D deficiency and their supplementation even for children and adolescents with 25D levels between 37.5-75 nmol/L to prevent bone disease in early adulthood [24]. This study may be helpful for further studies to identify mechanisms responsible for the low prevalence of secondary hyperparathyroidism in T1D subjects with low vitamin D levels.

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	Vitamin D deficiency level		
	Severe <12.5nM/L)	Moderate (12.6-25nM/L)	Mild 26-<50nM/L
Patients (84)	4 (4.76%)	17 (20.23%)	63 (52.92%)
Control (58)	2 (3.44%)	7 (12.06%)	49 (84.48%)

χ^2=1.88; d.f.-2; *P*=0.38

Table 3: Differences in proportions of patients and controls according to the vitamin D deficiency

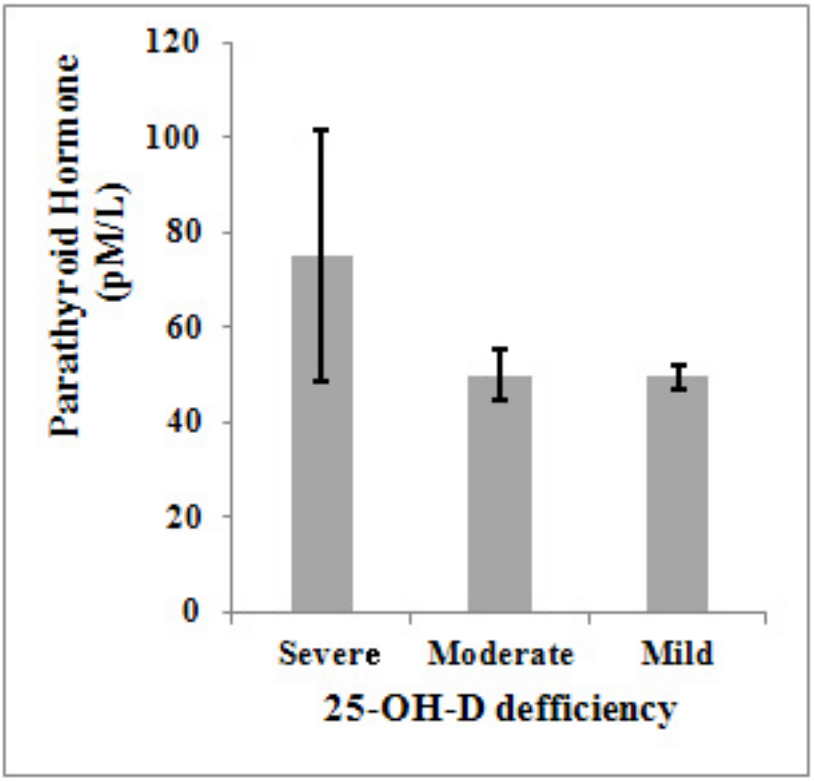

Figure 1: Parathyroid Hormone (mean±SEM) according to 25D levels-One-way-ANOVA showed significant difference between the means (P = 0.04). The graph shows that parathyroid hormone was significantly lower in patients with moderate and mild vitamin D deficiency compared to severely deficient patients.

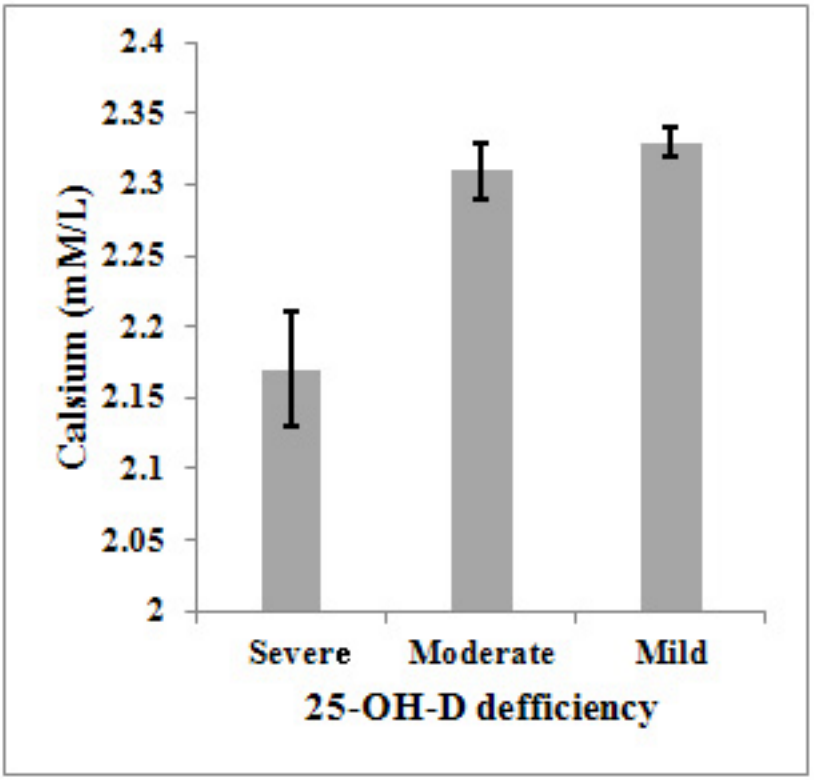

Figure 2: Serum calcium (mean±SEM) according to 25D levels-One-way-ANOVA showed significant difference between the means (P = 0.02). The graph shows that serum calcium was significantly lower in severely vitamin D deficient patients compared to patients with moderate and mild vitamin D deficiency.

Characteristics	Vitamin D deficiency level						P-value
	Severe (<12.5nMol/L)		Moderate (12.6-25nMol/L)		Mild (26-<50nMol/L)		
	Patients	Control	Patients	Control	Patients	Control	
Age	11.75 ± 0.85 a	10.0 ± 1.41 a	10.0 ± 0.53 a	9.14 ± 0.91 a	9.77 ± 0.42 a	8.51 ± 0.28 b	0.47
25-hydroxy vitamin D (nM/L)	9.25 ± 0.75 c	10 ± 0.0 c	21.82 ± 0.56 b	21.85 ± 0.98 b	36.31 ± 0.74 a	39.79 ± 0.80 a	<0.001
Parathyroid Hormone (pg/mL)	75.0 ± 26.65 a	35.0 ± 9 c	49.82 ± 5.45 b	80.14 ± 28.84 a	49.44 ± 2.31 b	52.81 ± 7.20 b	<0.05
Calcium (mM/L)	2.17 ± 0.04 b	2.35 ± 0.05 a	2.31 ± 0.02 a	2.31 ± 0.03 a	2.33 ± 0.01 a	2.32 ± 0.01 a	<0.05
Phosphate (mM/L)	1.47 ± 0.06 a	1.5 ± 0.1 a	1.57 ± 0.05 a	1.65 ± 0.07 a	1.51 ± 0.03 a	1.60 ± 0.03 a	0.66
Alkaline Phosphatase (Units/L)	322.75 ± 36.17 a	195.5 ± 10.5 c	282.44 ± 18.47 a	246.14 ± 22.63 b	266.17 ± 10.19 a	235.20 ± 9.94 b	<0.05
Body Mass Index	16.72 ± 1.16 a	16.8 ± 0.2 a	19.6 ± 1.11 a	19.2 ± 1.88 a	17.7 ± 0.43 a	17.92 ± 0.41 a	0.52
Duration of Type 1 diabetes (Months)	52.5 ± 22.89 a	NA	43.47 ± 6.4 a	NA	39.98 ± 4.39 b	NA	<0.05

Table 4: Comparison of mean of biochemical and physical characteristics of patients and control categorized according to vitamin D deficiency level

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