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Assessment of Vitamin D Status in Egyptian Children with Type-1 Diabetes Mellitus

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Abstract

Vitamin D and its active form are potent immune-modulators and are known to play an important role in the glucose/insulin metabolism. Studies on type 2 diabetes mellitus (T2DM) have shown that vitamin D increases insulin efficacy; However, there are no studies that define a similar relationship in type 1 diabetes mellitus (T1DM).

Aim of the work: To evaluate serum vitamin D status in children with type 1 diabetes mellitus with comparison to healthy subjects and to correlate its level with different clinical and laboratory parameters.

Subjects and methods: This study included two groups; Group I: included 53 patients with T1D; 43 patients with poor glycemic control (group Ia) and 10 patients with good glycemic control (group Ib). Another 15 apparently healthy children and adolescent's age and sex matched to the diseased group were taken as a control group and classified as group II. The studied groups were subjected to thorough history taking, clinical examination and laboratory investigations including: fasting blood glucose, glycated hemoglobin (HbA1c %), serum calcium level and 25-hydroxyvitD level. A serum 25OHD level of < 10 ng/mL was accepted as vitamin D deficiency, whereas < 20 ng/mL was accepted as vitamin D insufficiency.

Results: Serum 25-hydroxyvitD and serum calcium levels were significantly lower in T1D children than the control and in group Ia than group Ib. 25-hydroxyvitD had a significant strong positive correlation with serum calcium and significant strong negative correlations with fasting blood sugar and HbA1c %.

Conclusion: T1D children had significantly lower serum 25- hydroxyl vit D and serum calcium levels than the control group. Vitamin D in diabetic persons is an important factor in glycemic control with subsequent prevention of further complications.

Keywords: T1D; Vitamin D; Hemoglobin A1C

Introduction

T1D is an autoimmune disease, in which Beta cells of the pancreas are destructed resulting in a harmful state of hyperglycemia. One of the important factors that recent studies suggested that vitamin D deficiency has a role in the development of this disease. Vitamin D insufficiency is a risk factor for autoimmune diseases and other disorders. Although optimal supplement dosing with regard to immune and Beta cells function is not known. Vitamin D and its active form are potent immune-modulators and are known to play an important role in the glucose/insulin metabolism. Studies on T2DM have shown that vitamin D increases insulin efficacy; however, there are no studies that define a similar relationship in T1DM [1].

Aim of the work

To evaluate serum vitamin D status in children with type 1 diabetes mellitus with comparison to healthy subjects and to correlate its level with different clinical and laboratory parameters.

Subjects and methods

This study included 68 child and adolescent who were classified into two groups;

Group I: 53 patients who had already been diagnosed as diabetic patients according to the standard ADA criteria [2] and had regular follow up in pediatric endocrinology outpatients' clinic, Minia university children's hospital, Egypt and Group II: 15 apparently healthy subjects, age and sex matched to the diseased group. They were collected during the period from September 2012 to March 2013.

According to diabetic control and HbA1c % level, we divided the diabetic group into; group Ia (good controlled): they were 10 patients, their ages ranged between 7 and 18 years, 5 were males (50 %), and 5 were females (50 %), and their HbA1c level was up to 7 % and Group Ib (poor controlled): 43 patients, their ages ranged between 6 and 17 years, 21 were males (48.8 %), and 22 were females (51.2 %), and their HbA1c level was more than 7 %. The studied groups were subjected to the following: thorough history taking, clinical examination and laboratory investigations including: random blood glucose levels: (Colorimetric, Human, Germany):Assayed by using fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland) [2], HbA1c % as a parameter for glycemic control by using resin column chromatography. Kit contents were supplied by TECO DIAGNOSTICS, California; USA [3], Calcium Assessment (Colorimetric Method): with normal range of serum calcium in children:10-11.5 mg/dl [4], serum vitamin D(25-OH Vitamin D) determination by Immun-diagnostik Enzyme-Immuno-Assay (EIA) [5,6]. With normal range of 25-OH Vitamin D: 25-125

nmol/l. Classification of Vitamin D Status by 25(OH)D concentration [7]: ≤ 25 nmol/l (Deficient), >25 till 50 nmol/l(Insufficient)and >50 nmol/l (Optimal).

Statistical Methods [8]

The data were coded and verified prior to data entry. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS/Windows Version 19.0.0, SPSS Inc., an IBM Company). Microsoft excel 2013 was used for drawing figures. Continuous variables were presented as mean followed by standard deviation (SD), and categorical variables were presented as frequency and percentage. For qualitative data: Chi-square (X2) was used: For quantitative data: Independent-Samples T test (for two groups) used in person correlation for correlation between two quantitative variables, Two-tailed partial correlation coefficients (r) adjusted for age, sex and BMI was used to assess the relationships between 25-hydroxy vitD and other variables. P-value <0.001 (significant)and P-value >0.001(insignificant). Kendl's test: was used for correlation between quantitative and qualitative variables.

Results

Concerning clinical data, the diabetic children had significantly higher weight and lower height than the control group where (P<0.001) for both. On contrary, there were insignificant differences between them as regards BMI and waist circumference where (P=0.4) and (P=0.3) respectively (Tables 1 and 2) showed that diabetic children had significantly higher fasting blood sugar, HbA1c % and lower serum calcium and 25-hydroxyvitamin D than the control group where P<0.001 for all. Concerning 25-hydroxyvitD levels in diabetic patients, the current study found that 15.1 % of the studied diabetic group had deficient 25-hydroxyvitD levels while 84.9 % had insufficient 25-hydroxyvitD levels (N.B: Deficient ≤ 25 nmol/l, Insufficient >25 till 50 nmol/l (Figure 1). Regarding the glycemic control , the current study found that good glycemic controlled diabetic children had significantly lower fasting blood sugar and higher 25-hydroxyvitaminD levels than the poor glycemic controlled diabetic children where P<0.001 (Table 3). On contrary, there were insignificant differences between them as regard age, duration of DM, dose of insulin, type of feeding, family history of DM and serum calcium levels. Figure 2 showed that 3 patients with good glycemic controlled had deficient 25-hydroxyvitD versus 42 patients with poor glycemic controlled while 7 patients with good glycemic controlled had insufficient levels versus one patient with poor glycemic controlled. Concerning different correlations, Table 4 showed

Laboratory parameter	Group I No=53 (Mean ± SD)	Group II No=15 (Mean ± SD)	P- Value
Fasting blood sugar(mg/dl)	229.5 ± 92.6	92.8 ± 7.6	<0.001*
HbA1c (%)	8.7 ± 1.8	5.7 ± 0.6	<0.001*
Serum calcium(mg/dl)	8.7 ± 0.6	10.8 ± 0.5	<0.001*
25-hydroxyvitaminD (nmol/l)	19.1 ± 6.3	82.6 ± 15.8	<0.001*

*significant

Table 2: Comparison between the diabetic children and control group as regard some laboratory parameters.

Frequency of low 25-hydroxyvitamin D among the studied diabetic group

Figure 1: Frequency of low 25-hydroxyvitaminD among the studied group.
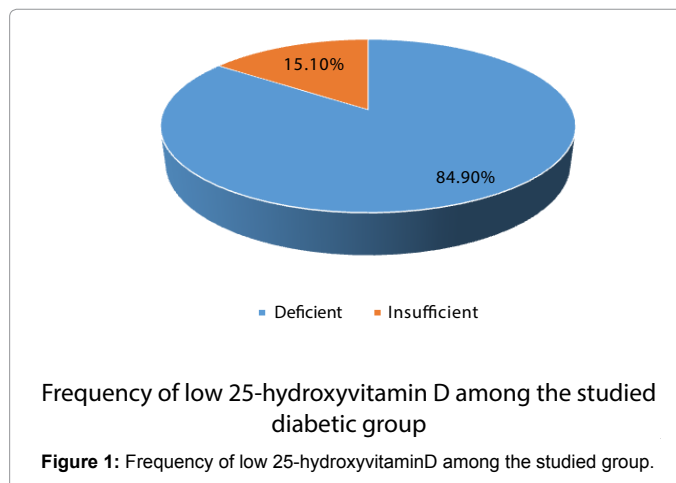
Parameter	Good controlled No=10	Poor controlled No=43	P- Value
Age:(year) 6-12. No (%) 5-10. No (%)	2(20%) 8(80%)	15(34.9%) 28(65.1%)	0.1
Duration of DM:(years) 3-5. No (%) >5. No (%)	8(80%) 2(20%)	37(86%) 6(14%)	0.6
Dose of insulin:(IU/kg/day) (Mean ± SD)	0.9 ± 0.14	0.9 ± 0.12	0.8
Type of feeding: Breast feeding. No (%) Artificial milk. No (%) Mixed. No (%)	7(70%) 0(0%) 3(30%)	39(90.7%) 1(2.3%) 3(7%)	0.1
Family history of DM: Positive. No (%) Negative. No (%)	2(20%) 8(80%)	9(20.9%) 34(79.1%)	0.9
Fasting blood sugar(mg/dl) (Mean ± SD)	121.2 ± 7.9	259.6 ± 75.5	<0.001*
Serum calcium level(mg/dl) (Mean ± SD)	8.8 ± 0.5	8.7 ± 0.6	0.7
25-hydroxyvitaminD (nmol/l): (Mean ± SD)	28.6 ± 6.5	16.9 ± 3.7	<0.001*

*significant

Table 3: Comparison between good and poor glycemic controlled diabetic children as regard some parameters.

that there were significant positive correlations between serum calcium with weight and 25-hydroxy vitamin D, while there were significant negative correlations between it with height, fasting blood sugar and HbA1c%. On contrary, there were insignificant correlations between serum calcium with BMI and insulin dose. Furth rely there were significant strong negative correlations between 25-hydroxyvitamin D and HbA1c % and fasting blood sugar where (r=-0.7, P<0.001) for each (Table 5). On the other hand, there was a significant strong

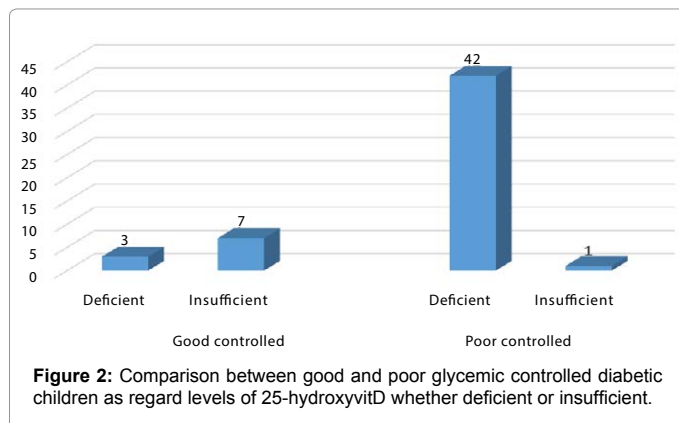| Clinical Parameter	Group I (Diabetic children) (N=53)	Group II (Control) (N=15)	P-Value
Age:(year) .6-12 years. No (%) .12-18 years. No(%)	17(32.1 %) 36(67.9 %)	5(33.3%) 10(66.6%)	0.6
Sex: Male. No (%) Female. No (%)	27(51 %) 26(49 %)	10(66.6 %) 5(33.4 %)	0.5
Weight (kg) (Mean±SD)	40.4 ± 16.2	37.9 12.6	<0.001*
Height (cm) (Mean±SD)	142.8 ± 21.1	149.3 ± 13.1	<0.001*
BMI (kg/m²)(Mean±SD)	18.9 ± 4.3	16.8 ± 2.7	0.4
Waist circumference (cm) (Mean±SD)	65 ± 10.9	68.1 ± 8.4	0.3

*significant

Table 1: Comparison between the diabetic children and controls as regard some clinical parameters.

Figure 2: Comparison between good and poor glycemic controlled diabetic children as regard levels of 25-hydroxyvitD whether deficient or insufficient.

Parameter	Serum calcium(mg/dl)	
	R	P
Weight (kg)	0.8	<0.001˙
Height (cm)	-0.8	<0.001˙
BMI (kg/m²)	-0.2	0.2
Fasting blood sugar (mg/dl)	-0.5	<0.001˙
25-hydroxyvitamin D (nmol/l)	0.8	<0.001˙
HbA1c (%)	-0.5	<0.001˙
Insulin dose (IU/kg/day)	-0.3	0.02

*significant
Grades of r:0.00 to 0.24 (weak or no association), 0.25 to 0.49 (fair association), 0.50 to 0.74 (moderate association), ≥ 0.75 (strong association)

Table 4: Correlations between serum calcium and some clinical and laboratory parameters among the diabetic children.

Parameter	25-hydroxyvitamin D	
	R	P
Age(year)	0.1	0.3
Gender	0.2	0.1
Duration of DM(year)	-0.1	0.2
Type of feeding	0.05	0.7
Dose of insulin(IU/kg/day)	-0.1	0.4
Weight(kg)	0.2	0.2
Height(cm)	0.1	0.5
BMI(kg/m²)	-0.2	0.1
Fasting blood sugar (mg/dl)	-0.7	<0.001*
HbA1c (%)	-0.7	<0.001*
Serum calcium (mg/dl)	0.8	<0.001*

Table 5: Correlations between 25-hydroxyvitamin D and some clinical and laboratory parameters.

positive correlation between 25-hydroxyvitamin D and serum calcium where(r=0.8, P<0.001) respectively.

Discussion

Type 1 diabetes mellitus (T1D) is an autoimmune disease in which the pancreas is unable to respond to secretagogue stimulation with appropriate insulin secretion. Hyperglycemia develops when more than 70-90% of the insulin-producing beta cells are destroyed. An autoimmune destructive process, which plays a central role in the development of T1D, is facilitated by the subject's own genetic susceptibility and by non-genetic factors. Non-genetic factors include viral infections, toxic chemicals, and others [9].

Vitamin D is a steroid hormone produced in the skin, ha specific

regulatory or functional effects on other parts of the body. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D (25[OH]D) and further hydroxylated in the kidney to 1,25-dihydroxyvitamin D. Hydroxylation in the kidney is regulated closely by parathyroid hormone (PTH), hypocalcaemia, and hypo-phosphatemia and is inhibited by 1,25-dihydroxyvitamin D [10]. Nutritional status with respect to vitamin D is best represented by the circulating concentration of plasma 25-hydroxyvitD [11].

The aim of our study was to evaluate vitamin D status in children with T1D and to correlate it with different clinical and laboratory parameters.

As regard the results of the current study, comparison between the diabetic children and the control group as regards some clinical parameters showed that group I had higher weight and shorter height than group II (Table 1). This result was in agreement with Thon et al. [12] and Paulino et al. [13] who found that diabetic children were higher in weight and shorter in comparison to healthy children. Concerning BMI and waist circumference, there were insignificant difference between group I and group II where P> 0.05. This result was in agreement with Mao et al. [14]. Regarding some laboratory parameters, Table 2 showed that group I had significant higher fasting blood sugar and HbA1c % than group II. This was in agreement with Paul et al. [15].

On the other hand, group I had significant lower serum calcium level than group II. This could be explained by that diabetic children had osmotic diuresis with glycosuria with subsequent increase of renal excretion of calcium (Hypercalciuria) which leads to reduction of serum calcium than the normal and good controlled patients [16].

Concerning 25-hydroxyvitamin D, group I had significantly lower serum level than group II where P<0.001. This could be explained by that Vitamin D deficiency may promote beta cells destruction in humans. Moreover, the presence of T1D is related to low vitamin D concentration whilst the lowering of 25-hydroxyvi tD levels during the years after diagnosis is a consequence of disturbed metabolism due to diabetes mellitus. Indeed, this may be explained why bone density is lower in T1D than in T2D [17]. In animal (mice) study, a vitamin D analogue had been shown to down regulate the production of pro-inflammatory chemokines , thereby inhibiting T-cell recruitment and the development of T1D [18].

Another possible mechanism is that vitamin D may have direct effects on B-cells, including improving insulin secretion, enhancing expression of vitamin D receptors and improving islet morphology [13]. This result was in agreement with Littorin et al. [19] who found that 25-hydroxyvitD was lower in patients with T1DM compared with control subjects whether they are recently diagnosed or after years of diagnosis. This finding may support the idea that vitamin D deficiency may be an important factor behind the development of T1D, perhaps with an immunological background [20-24]. Also, it was lower after years of diagnosis of T1D and this was in agreement with Svoren et al. [25] and Bin-Abbas et al. [26].

Figure 1 showed that 15.1 % of T1D patients were 25-hydroxyvitD insufficient and 84.9 % were deficient. In comparison to our result, Bener et al. [27] found 90.6 % of diabetics versus 85.3 % of non-diabetics had vitamin D deficiency and in northeastern US , it had been found that 15 % of T1D were 25-hydroxyvitD deficient versus 61 % insufficient [28]. Furthermore, Janner et al. [29], found 60.5 % of diabetic children were vitamin D deficient. Similarly, in North India, in a case-control study, 58 % of T1D versus 32 % of the control [30].

Recently, Piccini et al. [31] found that vitamin D deficiency was present in 43.9 % of patients and insufficiency in35.5 % according to studies conducted on healthy children. Furthermore, Branco et al. [32] found that there was insufficiency in 28 % of T1DM patients, deficiency in 32 %, severe deficiency in 9 % and normal in 31 %, 55 % deficiency of boys and 45 % of girls. Concerning glycemic control, Table 3 showed that good glycemic controlled children had insignificant difference than poor glycemic controlled children as regard age and duration of DM. This was in agreement with Hartmann et al. [33]. As regard the dose of insulin, there was insignificant difference between good and poor glycemic controlled patients where (P=0.3). This was in agreement with Reinhard et al. [34] who found that metabolic control was unsatisfactory in many patients with T1D irrespective of the insulin regimen. Regarding the type of feeding, the current study found insignificant difference between good and poor glycemic controlled children where (P=0.1). This finding was in contrary to the finding by Hana et al. [35]. Concerning family history of DM, there was insignificant difference between good and poor glycemic controlled children where (P=0.9) (Table 4). This was in contrary to Lucy et al. [36] who found that a higher familial risk of diabetes had a worse glycemic control. Furthermore, this was in contrary to Kuo et al. [37] who found that positive paternal history was associated with worse glycemic control.

As regard calcium levels, there was insignificant difference between good and poor glycemic controlled children where (P=0.7). This was in agreement with Alberto et al. [38] while others such as Duarte et al. [39], Blasiak et al. [40] found significant difference between good and poor glycemic controlled children

Concerning 25-hydroxy vitD, good glycemic controlled children had significant higher levels than poor glycemic controlled children where (P<0.001).

Concerning different correlations, Table 4 showed that there were significant correlations between serum calcium and weight, height, fasting blood sugar, 25-hydroxyvitD and HbA1C % where (P<0.001). In contrary, there were insignificant correlations between calcium with BMI and insulin dose.

Table 5 showed that there were significant strong negative correlations between 25-hydroxyvitD and fasting blood sugar, HbA1c % where (r=-0.7, P<0.001) respectively. In contrast, Littorin et al. [19] found that there was insignificant correlation between 25-hydroxy vit D levels and HbA1c % and this indicated that the diabetic state per say is a reason for low 25-hydroxyvitD levels and is not secondary to any hyperglycemic or insulin-resistant state. On the other hand, there was a significant strong positive correlation between 25-hydroxyvitD and serum calcium where (r =0.8, P<0.001) respectively.

Recommendations

Screening for vitamin D deficiency, insufficiency and supplementation in children with T1D and groups at risk for this disease. There is a need to increase the dose of vitamin D supplemented to diabetic children to avoid many complications. Follow up studies of supplemented diabetic children with vitamin D deficiency and evaluation of their glycemic control.

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