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Vitamin D status in Egyptian children with type 1 diabetes and the role of vitamin D replacement in glycemic control

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Abstract

Background: The association of low serum 25 hydroxy cholecalciferol (25(OH)D) levels with high glucose level and diminished insulin sensitivity suggests that vitamin D (VD) may modulate insulin metabolism. The aim of the study was to screen for vitamin D deficiency (VDD) in pediatric patients with type 1 diabetes (T1D) and study the effect of VD supplementation on their glycemic control and insulin requirements.

Methods: A prospective cohort study including 50 patients with T1D. VD level was assessed initially and after 3 months of VD supplementation (in those with VDD). HbA1c and insulin requirements were studied at 0, 3 and 6 months of supplementation.

Results: Fifty patients with T1D were included with mean diabetes duration of 4.11 ± 2.34 years. VD level ranged from 0.2 to 33 ng/mL. VD status correlated significantly with daily insulin dose (p = 0.030, r = 0.306) and HbA1c (p < 0.001, r = 0.243). Thirty-five patients (70%) had VDD and were allocated for VD supplementation for 3 months. The mean HbA1c improved significantly after supplementation (p = 0.003), followed by a significant deterioration at 6 months with no change in their insulin requirements at 3 or 6 months.

Conclusions: VD was highly prevalent in Egyptian T1D patients. VD supplementation improved glycemic control at 3 months after therapy with no reduction in insulin requirements.

Keywords: glycemic control; type 1 diabetes; 25(OH)D; vitamin D supplementation.

Introduction

High prevalence of vitamin D deficiency (VDD) in children and adolescents with type 1 diabetes (T1D) has been reported in several epidemiological studies suggesting an association between both [1, 2]. Data showed that T1D patients had lower levels of 25 hydroxy cholecalciferol [25(OH)D] than controls [3] and that exposure to sunlight early in life [as a source of vitamin D (VD)] can prevent development of T1D [4]. VD has anti-inflammatory and immune-modulatory effects that could influence the autoimmune pathology of T1D [5]. VD may have a role in the Th1-mediated autoimmunity against pancreatic β-cells causing their destruction [6]. There are significant higher insulin requirements in T1D patients with VDD together with low insulin sensitivity [7], higher fasting glucose and higher levels of glycosylated hemoglobin [8]. Increasing vitamin D levels from 25 to 75 nmol/L was shown to improve insulin sensitivity by 60% [9]. VDD has been involved in the development of diabetes complications as nephropathy and retinopathy [10–12].

VDD is highly prevalent in children and adolescents with T1D, but is underestimated, thus screening and supplementation should always be considered [13]. VD supplementation is probably necessary for most patients with T1D as diet alone will not provide sufficient amounts of VD [14]. Cholecalciferol, being safe and inexpensive might become an adjuvant in combination with insulin to control T1D [15]. Also, VD supplementation may have a role in preserving residual β cell function [16]. The aim of the current study was to screen for VDD in pediatric patients with T1D and to study the effect of VD supplementation on glycemic control and insulin requirements in those patients.

Materials and methods

This prospective cohort study included 50 patients with T1D above 5 years of age with onset of T1D >1 year, with no hepatic or renal problems or any drug therapy that may affect VD metabolism and after exclusion of celiac disease, enrolled during their regular follow-up visits to the Diabetes, Endocrine and Metabolism Paediatric Unit (DEMPU) outpatient clinic at Cairo University (April 2014 to December 2014) aiming at the assessment of VD status and studying the effect of VD supplementation on glycemic control. 25-Hydroxyvitamin D [25(OH)D] level
was assessed initially and after 3 months of vitamin D3 supplementation (in those with VDD) in a dose of 4000 IU/day in addition to oral calcium supplementation in a dose of 50 mg/kg/day. Glycemic control (HbA1c) and insulin requirements were studied at 0, 3 and 6 months of VD therapy. The study protocol was approved by the Research Ethics Committee of Cairo University and was in accordance with the 1964 Helsinki declaration and its later amendments, and patients were included after obtaining informed consents from their legal guardians.

PTH levels were measured with DRG ELISA kit (DRG International, Inc., Germany). The DRG Intact PTH Immunoassay is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of the biologically intact 84 amino acid chain of PTH [17]. The HbA1c% measurement is based on a modification of the alkaline hematin reaction. Using the values obtained for each of these two analytes in (g/dL), the percentage of the total hemoglobin that is glycated is calculated and reported as %HbA1c. The final HbA1c% result has been standardized to the results obtained in the Diabetes Control and Complications Trial (DCCT). A 25(OH) D assay was done by immunochemiluminescence assay on a Siemens ADVIA Centaur (USA) [18]. VDD was considered with 25OHD <11.7 ng/mL in males, <14.3 ng/mL in females. VD insufficiency was considered with 25OHD <20 ng/mL and VD sufficiency with 25OHD ≥20 ng/mL. Data was statistically described in terms of mean ± standard deviation (±SD), and range. Comparison of numerical variables over the study time points was done using a paired t-test in comparing two groups and repeated measure analysis of variance through a general linear model analysis when comparing more than two groups. Correlation between variables was done using a Pearson's correlation equation for linear relation in normally distributed variables and a Spearman’s rank correlation equation for non-normal variables/non-linear monotonic relation, p-Values <0.05 was considered statistically significant. Statistical software used was SPSS (Statistical Package for the Social Sciences, IBM, Armonk, NY, USA).

Results

This study included 50 patients (23 females and 27 males) with T1D with a mean age of 10.24 ± 3.46 years. Their age at onset of diabetes ranged from 1 to 14 years with a mean of 6.16 ± 3.4 years, whereas their diabetes duration range was 1.3–11.5 years of mean 4.11 ± 2.34 years. Vitamin D (25OHD) level ranged from 0.2 to 33 ng/mL with a mean of 11.246 ± 5.716 ng/mL. Most of our patients lived in urban areas (76%) in Cairo or its vicinity.

Correlation of VD status (degree of VDD) of the 50 patients with other parameters (before supplementation) revealed a significant positive correlation with insulin daily dose (p = 0.030, r = 0.306), serum magnesium levels (p = 0.049, r = 0.280) and HbA1c (p = 0.00, r = 0.243) (Table 1). However, no significant correlation was detected between VD level and the duration of diabetes, insulin dose or HbA1c%. Among the studied patients, 94% (n = 47) were VD deficient or insufficient. Thirty-five patients (70%) were VD deficient, 12 (24%) had VD insufficiency and only three patients (6%) were sufficient for VD. Males had higher mean values of 25OHD serum levels (12.66 ± 6.35 ng/mL) compared to females (9.578 ± 4.43 ng/mL). Also 91.3% of females had VDD compared to 51.9% of the males (Figure 1). The VDD group (n = 35) had a significantly higher mean values of daily insulin requirements (p = 0.042) compared to VD insufficient (n = 12) or sufficient (n = 3) groups, but the three groups were comparable in their HbA1c mean values (Table 2). When the patients were divided into three groups according their HbA1c% status; as being optimal (≤7%), sub-optimal (7–9%) and high risk (>9%), a significant difference was found in their 25OHD mean levels (p = 0.036); with the high risk group having significantly lower mean 25OHD levels compared to the optimal and sub-optimal groups (Figure 2) VD deficient patients (n = 35) were allocated to VD supplementation for 3 months, two of them were excluded from the study due to non-compliance to therapy. All the treated patients (n = 33) had significant improvement in their 25OHD levels (p = 0.000). No statistically significant difference was found regarding their insulin requirements after 3 and 6 months of supplementation (p > 0.05). However, their mean HbA1c decreased from 9.413 ± 1.978% to 8.785 ± 1.583% after 3 months of supplementation, showing a statistically significant improvement

| Table 1: Correlation of vitamin D status with biochemical tests, insulin requirements and HbA1c pretreatment. |
|---|---|---|
| VD status | r-Value | p-Value |
| ALP pretreatment | −0.098 | 0.499 |
| Calcium pretreatment | −0.105 | 0.468 |
| Phosphorous pretreatment | −0.094 | 0.516 |
| Magnesium pretreatment | 0.280 | 0.049 |
| Insulin dose pretreatment | 0.306 | 0.030 |
| HbA1c, % pretreatment | 0.243 | 0.001 |

Bold values indicates statistically significant (p < 0.05).

Figure 1: Gender difference in relation to vitamin D status (p = 0.009).
Table 2: Comparison between the three groups of vitamin D regarding their demographic parameters, biochemical tests, HbA1c and insulin dose mean levels before supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D deficient (n = 35)</th>
<th>Vitamin D insufficient (n = 12)</th>
<th>Vitamin D sufficient (n = 3)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>10.33 ± 3.56</td>
<td>9.95 ± 3.54</td>
<td>10.38 ± 2.79</td>
<td>0.94</td>
</tr>
<tr>
<td>Age at onset, years</td>
<td>6.22 ± 3.39</td>
<td>5.76 ± 3.9</td>
<td>7 ± 1.22</td>
<td>0.83</td>
</tr>
<tr>
<td>Diabetes duration, years</td>
<td>4.14 ± 2.5</td>
<td>4.2 ± 2</td>
<td>3.36 ± 1.6</td>
<td>0.85</td>
</tr>
<tr>
<td>BMI, SDS</td>
<td>0.7 ± 0.77</td>
<td>0.9 ± 0.88</td>
<td>0.7 ± 0.52</td>
<td>0.73</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.1 ± 0.76</td>
<td>9.2 ± 0.66</td>
<td>9.4 ± 1.15</td>
<td>0.77</td>
</tr>
<tr>
<td>Phosphorus, mg/dL</td>
<td>4.4 ± 0.6</td>
<td>4.33 ± 0.59</td>
<td>5 ± 0.85</td>
<td>0.20</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>25.7 ± 34.6</td>
<td>21.24 ± 15.2</td>
<td>20.2 ± 5.1</td>
<td>0.88</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>194.2 ± 76.8</td>
<td>200 ± 67.3</td>
<td>227 ± 62.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Magnesium, mg/dL</td>
<td>2.2 ± 1.14</td>
<td>2.1 ± 0.25</td>
<td>1.93 ± 0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Insulin dose, IU/kg/day</td>
<td>1.2 ± 0.38</td>
<td>0.9 ± 0.29</td>
<td>0.99 ± 0.16</td>
<td>0.042*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>9.38 ± 1.99</td>
<td>8.2 ± 1.69</td>
<td>8.56 ± 0.49</td>
<td>0.17</td>
</tr>
</tbody>
</table>

BMI, body mass index; PTH, parathormone; ALP, alkaline phosphatase; SDS, standard deviation score. *p < 0.05.

Figure 2: HbA1c in the study group.

Discussion

This study evaluated the prevalence of VD deficiency in a group of Egyptian children and adolescents with T1D aiming to study the effect of VD supplementation on their glycemic control. It was found that 70% of the patients had VDD, 24% had insufficiency and only 6% were VD sufficient. This high prevalence of VDD in patients with T1D was previously reported in Egypt. In Cairo University, Hassan et al. [13] enrolled 60 children and adolescents with T1D to assess their VD levels and concluded that 91.67% were VD deficient. Similarly, in Menia and Zagazig, studies revealed a prevalence of 84.9% and 75%, respectively [19, 20]. This high prevalence of VDD in Egypt despite the presence of adequate sunlight and sunny weather most of the year can be attributed to the
VD receptor gene polymorphism [21] as well as the lack of skin exposure to sunlight due to cultural aspects. Globally, VDD prevalence was found to be highly variable in other studies, ranging from as low as 15% to as high as 90.6% [1, 2, 22–25].

Many observational studies, assessed the 25OHD levels in children and adolescents with T1D in different countries. Egypt was found to be in the fourth rank regarding the mean 25OHD levels (18.7 ng/mL) following Sweden, Australia and USA [26]. Greer et al. [27] concluded that even in environments with abundant sunlight, children and adolescents with T1D have lower 25OHD levels raising the question whether VDD should be routinely screened for and VD supplementation given to those with low levels.

In the current study, no significant sex difference was found regarding calcium homeostasis parameters, insulin daily requirements or HbA1c% but males had higher mean 25OHD levels. This could be attributed to females covering their head and body due to cultural and religious reasons. The more VD requirements for bone growth during faster pubertal spurt in females [28] could be an additive factor. That was also the case in the study conducted by Al-Agha and Ahmad in Saudi Arabia; where the boys had higher levels of VD compared to the girls [29]. Similarly, Ataie-Jafari et al. [30] in Iran found a significant difference between males and females in 25OHD levels, the same as Neyestani et al. [31]. In contrast, Branco et al. [32] and Mutlu et al. [33] found no significant difference between males and females regarding 25OHD, calcium homeostasis or their glycemic control. Surprisingly, Ordooei et al. [34] found a significantly lower mean levels of 25OHD and phosphate in males compared to females.

When the patients were divided according to their VD levels into three groups (deficient, insufficient and sufficient), it was found that though all groups were comparable in their mean age, diabetes duration, BMI SDS. The VDD group (n=35) had a significantly higher daily insulin requirements when compared to the insufficient and sufficient groups. The lower insulin requirements in the sufficient group might indicate increased insulin sensitivity in those patients [7]. Janner et al. [2] showed no significant difference in mean HbA1c% between VD deficient and sufficient groups as did Nwosu et al. [35]. On the other hand, Janisse et al. [36] found that adolescents with low VD intake had higher HbA1c%, as did Al-Agha and Ahmad [29].

The present study showed no difference between the 3 VD groups in the mean age or duration of T1D. On the contrary, Svoren et al. [1] in USA and Vojtkova et al. [37] in Slovakia reported that VDD patients were significantly older and had longer diabetes duration than non-deficient patients. Azab et al. [38] found no significant correlation between 25OHD and age, calcium homeostasis or HbA1c% but a significant positive correlation between 25 OHD and diabetes duration as did Svronek et al. [1], whereas Bin-Abbas et al. [39] found an inverse significant correlation between 25OHD and diabetes duration.

A significant positive correlation was found between the severity of VDD and the daily insulin requirements and also with their HbA1c%. Similarly, Al-Agha and Ahmad [29] identified a significant relationship between HbA1c and the status of VD among the selected diabetic patients. Tunc et al. [7] in Turkey found a weak negative correlation between 25OHD level and the daily insulin dose. Soliman et al. [19] found a significant strong inverse correlation between 25OHD levels and HbA1c% and between 25OHD and calcium levels. This was also concluded by Aljabri et al. [40]. On the other hand, Janner et al. found no such significant correlation in the Swiss population [2].

In this study, VD supplementation was associated with a significant improvement in HbA1c% mean values, which was not sustained 3 months later, whereas the daily insulin requirements did not show any significant change after VD treatment. The deterioration in HbA1c at 6 months (after the initial improvement) can be explained by the fact that the patients did not receive maintenance dose of VD after the 3 months of therapy. Similarly, Parildar found a significant reduction in HbA1c insulin and HOMA-IR following VD supplementation in patients with prediabetes favoring the improvement in glucose metabolism [41]. Aljabri et al. [40] concluded that VD supplementation might improve the glycemic control of patients with T1D as there was significant reduction in HbA1c% levels following a supplementation given to 80 patients with T1D aged over 12 years with 25OHD <20 ng/mL, but there were no data if that effect would be sustained further, which was the case in this study. Nwosu and Maranda reported an initial, clinically-significant reduction in HbA1c% in patients with T2D, but not T1D after VD supplementation. Thus, it is possible that VD alone has insignificant role in improving glycemic control in patients with T1D [42]. Mohammadian et al. [25] found that after VD supplementation in T1D patients with VDD, the HbA1c% mean levels improved significantly and also the patients were transferred towards better glycemic control across the three groups of HbA1c% [28] unlike the present study which did not show significant change.

On the other hand, Bizzarri et al. found that VD supplementation was ineffective in reducing insulin requirements in patients with newly diagnosed T1D. They concluded that daily calcitriol had no effect on residual β cell function, insulin dose or glycemic control in children and adolescents with T1D of recent onset. As at 6, 12 and 24 months after VD supplementation; their insulin
requirements did not differ from those treated with placebo [43].

Walter et al. [44] as well as a meta-analysis conducted by George et al. [45] in Scotland, observed no significant improvement in HbA1c % or fasting blood glucose in those treated with VD supplementation compared with those who received placebo concluding that there was insufficient evidence of the beneficial effect of VD supplementation as a means of improving glycemic control in children and adolescents with T1D. Further studies are needed to study the effect of long term supplementation of VD as well as serial assessment of VD level to judge the role of VD supplementation in glycemic control in T1D patients.

## Conclusions

A high prevalence of VDD (70%) and insufficiency (24%) was found among Egyptian children and adolescents with T1D. Patients with VDD had higher daily insulin requirements and higher HbA1c levels. HbA1c showed a significant improvement after 3 months of vitamin D and calcium supplementation which was not sustained 3 months later while daily insulin requirements did not show any change in response to VD supplementation.

### Author contributions:
All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. Mona Hafez and Mona Hassan set the study design. Sally Abdel Azim and Noha Musa recruited the patients, collected the samples and supplemented the patients with vitamin D. Sahar Sharaf performed the laboratory assessment. Sally Abdel Azim collected and tabulated the data. Noha Musa, Mona Hafez and Mona Hassan wrote the paper.

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