

# Impact of Vitamin D supplementation on lipid profile in children and adolescents with type 1 diabetes

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## ABSTRACT

**Background:** Type 1 diabetes (T1D) is a chronic childhood disorder and is likely to be associated with dyslipidemia. Vitamin D deficiency further increases the risk of dyslipidemia; Vitamin D is believed to have cardioprotective properties. **Objectives:** The objective of this study was to study the effect of Vitamin D supplementation on lipid profile and glycemic control (hemoglobin A1c [HbA1c]) in children with T1D. **Materials and Methods:** A prospective, non-blinded, single-arm, supplementation study was performed in 145 underprivileged children with T1D from 2015 to 2017. Anthropometry, biochemical parameters (HbA1c, Vitamin D, and lipid parameters) were assessed at baseline. Children were supplemented with 1000 IU Vitamin D and 500 mg calcium daily. Follow-up data at the end of 1 year of supplementation were recorded. **Results:** The mean age at presentation of T1D in children was 12.5±3.9 years (65 boys). Baseline HbA1c, cholesterol, and Vitamin D were 10.3±2.1%, 4.1±1.1 mmol/L, and 33.6±18.5 nmol/L, respectively. At end line, HbA1c, cholesterol, and Vitamin D were 9.4±1.7%, 3.3±0.8 mmol/L, and 51.1±21.8 nmol/L, respectively (p<0.05 for all). Regression analysis showed that increase in Vitamin D was associated with improvement in cholesterol and HbA1c (p<0.05). **Conclusion:** Daily supplementation with Vitamin D along with calcium in children with T1D results in improvement in lipid profile and glycemic control.

**Key words:** Dyslipidemia, India, Type 1 diabetes, Vitamin D supplementation

Type 1 diabetes (T1D) is a common chronic childhood disorder with increasing prevalence in most countries including India [1]. Children with diabetes are known to have impaired lipid metabolism due to chronic hyperglycemia [2]. Impaired lipid metabolism is further likely to lead to cardiovascular complications in children with diabetes which can be a major cause of morbidity and mortality [3].

Vitamin D deficiency is common in general population; however, reports suggest that its prevalence is higher in children and adolescents with diabetes than in general population [4-6]. Vitamin D is believed to stimulate insulin receptor expression and also insulin-induced glucose transport *in vitro*; hence, its low concentrations are associated with impaired insulin sensitivity [7]. It also regulates fatty acid metabolism in skeletal muscle and adipose tissue, and previous work suggests that its deficiency increases the risk of dyslipidemia [8].

Since Indian children with diabetes are likely to have Vitamin D deficiency and are also likely to have deranged lipid profile [3,9], we conducted a study on children with diabetes with primary objective to study the effect of Vitamin D supplementation on lipid profile and secondary as on glycemic control (hemoglobin A1c [HbA1c]) in children with T1D.

## MATERIALS AND METHODS

This was a prospective, non-blinded, single-arm, supplementation study conducted over 15 months at a tertiary level care pediatric endocrine unit in Western Maharashtra from January 2015 to June 2017. The inclusion criterion was children between 3 and 18 years of age with T1D who were on treatment, with disease duration of more than 1 year. Children with associated disorders such as celiac disease, hypothyroidism, and other autoimmune conditions were excluded. The institutional ethics committee approved the study protocol and parents gave written informed consent and children assent (as appropriate) for the study.

Data pertaining to age (date of birth), gender, medical history, insulin dosage, date of diagnosis of T1D, and anthropometry (height in centimeters, weight in kg, and body mass index [kg/meter square]) [10] were noted. Data regarding parent's education and family income were also collected and used to assess socioeconomic status of the family using the Kuppuswamy's method [11]. Biochemical tests were performed after an overnight fasting (of more than 12 h) between 7 and 10 AM in the morning; 6 ml of blood was drawn under strict aseptic precautions using plain and ethylenediaminetetraacetic acid

vacutainers (BD Franklin Lakes, NJ, USA). Serum was separated after centrifugation at 2500 rpm for 15 min at room temperature within 2 h of collection.

Tests performed included glycosylated hemoglobin (HbA1c by high-performance liquid chromatography method), high-density lipid cholesterol (HDL-C), total cholesterol (TC), and triglyceride (TG) (measured by enzymatic method). Low-density lipoprotein cholesterol (LDL-C) and very LDLC concentrations were calculated using Friedewald equation. Vitamin D, i.e., 25 (OH) D concentrations was estimated by enzyme-linked immunosorbent assay (Developmental language disorder Diagnostics GmbH, intra-assay coefficients of variation (CV), 4.9; interassay CV, 7.8%). Parathyroid hormone (PTH) concentration was measured by chemiluminescent immunoassay (Siemens, India). Vitamin D levels were classified as sufficient >50 nmol/L, insufficient 30–50 nmol/L, and deficient <30 nmol/L [12].

However, extraskelatal benefits of Vitamin D have been observed at concentrations of >45 ng/ml (>112 nmol/L) [13]. Bone profile included serum calcium, phosphorus, alkaline phosphatase (STAR 21, colorimetric method, interassay variation <5%), and PTH (ELISA, Bio-Rad-iMark, interassay variation <10%). Lipid parameters were classified for dyslipidemia [14] (high TC >200 mg/dL, high TGs >100 mg/dL, high LDL >130 mg/dL, and low HDL <40 mg/dL).

Sunlight exposure was assessed using structured validated questionnaire [15]. Information on sunlight exposure in minutes (between 7 AM and 11 AM, 11 AM and 3 PM, and 3 PM and 7 PM), mode of travel and transport, clothing pattern, use of cap or helmet, and sunscreen use was noted [16]. Data on physical activity were collected by interview method using the QAPACE questionnaire for children that were adapted for Indian children [17]. Time spent in sports activities was considered as moderate activity [18].

Dietary data were collected using a 24-h recall on 3 non-consecutive days of week, through a face-to-face interview using multiple-pass method. Nutrient intakes were calculated using C-DIET version 2.0 (which comprises nutritive values of cooked foods and raw foods database of National Institute of Nutrition, India) [19]. Carbohydrate density, i.e., the amount of carbohydrate consumed per 1000 kcal was calculated using the formula: Carbohydrate density = (Total carbohydrate intake/total calorie intake)×1000. Similarly, fat density and zinc density were also computed [20].

All children were supplemented with Vitamin D, 1000 IU daily as an oral capsule, which was lipid soluble, and in gel form, soft capsule, non-chewable, advised to be taken after meal once daily. The dose of 1000 IU was decided based on the upper safe limit of Vitamin D in children as per the American Association of Pediatric Guidelines, 2011 [21]. Children and parents were instructed not to chew the capsule. As the previous studies show that underprivileged children have poor calcium intake [22], children were also supplemented with 500 mg of elemental calcium daily.

Children were called to the center monthly and tablets for the next month were dispensed and empty packets were collected. A percentage of intake and distributed capsule was calculated.

Compliance was assessed by regular phone calls, weekly text messages, and in person during follow-up at every monthly visit. No adverse events such as hypercalciuria (urinary spot calcium and creatinine ratio) or signs of Vitamin D toxicity related to the supplementation were observed. After 1 year of supplementation, these children underwent repeat assessment of all above parameters. Percentage change in HbA1c, cholesterol, and Vitamin D was computed.

All data were entered into Microsoft Excel (version 2015) and were analyzed using IBM SPSS version 25 (Bengaluru, India). Normality of data was checked and normal variables were described as mean ± standard deviation and non-normal variables as median (interquartile range), (non-normal variables included PTH, phosphorus, physical activity, and sunlight exposure). Paired t-test was used to compare differences in baseline and end line parameters among normally distributed parameters. For non-normally distributed variables, non-parametric tests were used. Multiple logistic regression analysis was performed to assess improvements in TC and HbA1c. In Model I and II, HbA1c and Vitamin D were the independent variables while in Models III and IV, physical activity, dietary factors, and sunlight exposure were independent variables. Disease duration was included in all models. Based on earlier literature [2,7], for 20% reduction in mean cholesterol concentrations for achieving 80% power at 5% level of significance, the estimated sample size was 130 subjects.

## RESULTS

A total of 145 children were enrolled in the study and 136 completed 1 year supplementation. Baseline characteristics of subjects who did not complete the study were similar to the 136 subjects who completed the study ( $p>0.1$ ). Mean age at enrollment was 12.5±3.9 years (65 boys and 71 girls). The baseline and end line anthropometric and lifestyle parameters are presented in Table 1. Results were similar in both boys and girls ( $p>0.1$ ). The

**Table 1: Baseline and end line anthropometry and lifestyle parameters**

Parameter	Total (n=136)	
	Baseline	End line
Age (year)	12.5±3.9	13.5±3.9
Height (cm)	140.8±18.1	144.8±17.7
Height Z score	-0.5±1.5	-0.4±1.9
Weight (kg)	36±14.1	39.9±14.1
Weight Z score*	-0.5±1.1	-0.3±1
BMI	17.4±3.7	18.8±7.5
BMI Z score	-0.3±0.9	-0.2±1.1
Waist Z score*	-0.9±2.1	-1.2±1
Disease duration (year)	4.5±3.5	5.5±3.6
Insulin requirement (IU/kg/Day)	1±0.4	1.1±0.3
Boys:Girls	65:71	65:71
Moderate activity (min/week)*	213±252	164.4±240.9
Effective sunlight exposure (min/week)	49.6±41.3	49±31.9

(\*—  $p<0.05$ ). BMI: Body mass index

average compliance of the supplementation was around 90% in the children. At baseline 35, 27, 15, 4, and 55 were in pubertal stage 1, 2, 3, 4, and 5, respectively. At end line, the pubertal status of the study population was 26, 20, 20, 7, and 61 in stage 1, 2, 3, 4, and 5, respectively. During the study period, 19.28 episodes per 100 patient-years of diabetic ketoacidosis were observed.

Biochemical parameters showed significant improvement in HbA1C ( $p<0.001$ ) and bone profile including serum alkaline phosphatase, Vitamin D [25(OH) D], PTH, and phosphorus ( $p<0.05$  for all). However, serum calcium did not change significantly. Lipid profile showed lowering of total serum cholesterol, HDL, and LDL ( $p<0.05$ ), whereas TG ( $p<0.001$ ) and cholesterol:HDL ( $p<0.05$ ) increased. Percentage of children with total and LDL cholesterol within reference range was higher at end line than at baseline (97.8% vs. 91.1%); although the TG concentrations increased, the percentage within reference range remained similar (91.9% end line and baseline 89.7%). The details are mentioned in Table 2.

At baseline, of 136 children, 57 (41.9%) were Vitamin D deficient, 54 (39.7%) were insufficient, and 25 (18.4%) were sufficient. At the end line, 17 (12.5%) were deficient, 49 (36%) were insufficient, and 70 (51.5%) children were sufficient. The mean HbA1c was not different in the groups with deficient, insufficient, and sufficient Vitamin D levels at end line. However, reduction in cholesterol was significantly higher (23%) in the sufficient group versus the insufficient and deficient group (13% and 11%, respectively).

In the regression analysis, Model I showed that there was a significant improvement in Vitamin D with improvement in cholesterol concentrations ( $p<0.05$ ). Model II showed that HbA1c improved along with improvement in cholesterol concentrations ( $p<0.05$ ). Model III showed that carbohydrate density in the diet was associated with improvement in cholesterol concentrations ( $p<0.05$ ). Model IV showed that diet and activity did not influence HbA1c improvement. The details are mentioned in Table 3.

**Table 2: Baseline and end line biochemical parameters (\*- $p<0.05$ )**

Parameter	Total (n=136)	
	Baseline	End line
Alkaline phosphatase (IU/L)*	245.9±96.7	148.7±75.9
HbA1C (%)*	10.3±2.1	9.4±1.7
Vitamin D (nmol/L)*	33.6±18.5	51.1±21.8
PTH (ng/L)*	58.5 (43)	44 (37.5)
Serum calcium (mmol/L)	2.3±0.7	2.4±0.2
Serum phosphorus (mmol/L)*	1.5±0.2	1.2±0.9
Urinary spot calcium creatinine ratio (mg/mg)	0.1±0.09	0.13±0.1
Cholesterol (mmol/L)*	4.1±1.1	3.3±0.8
HDL (mmol/L)*	1.3±0.3	1±0.4
LDL (mmol/L)*	2.4±1	1.8±0.8
Triglyceride (mmol/L)*	0.8±0.4	1±0.4
VLDL (mmol/L)	0.4±0.2	0.4±0.2
Cholesterol: HDL*	3.2±0.8	3.5±1

HDL: High-density lipid, LDL: Low-density lipoprotein, PTH: Parathyroid hormone, HbA1C: Hemoglobin A1c, VLDL: Very low-density lipoprotein

## DISCUSSION

Our results suggest that 1 year daily supplementation with 1000 IU Vitamin D and 500 mg calcium in underprivileged children with T1D resulted in a reduction in waist circumference and HbA1c, and improvement in the bone profile. Although mean HDL reduced, low HDL concentrations have been reported in healthy Indian children (lower than 1 mmol/L) [23].

We found that Vitamin D with calcium supplementation in children with T1D resulted in a decrease in TC and LDL concentrations. Similar results have been reported in adults with type 2 diabetes mellitus in a recent meta-analysis by Jafari *et al.* [24]. Cholecalciferol (Vitamin D3) and 25(OH) D inhibit HMG-CoA reductase activity (a rate-limiting enzyme in cholesterol synthesis) in human skin fibroblasts and hence lower cholesterol concentrations [25]. We also found that carbohydrate density in the diet was associated with cholesterol concentrations. It is suggested that there is an unfavorable impact on lipid profile with increase in carbohydrate intake [26]. Reduction in HDL cholesterol after supplementation with Vitamin D has also been previously reported [24], which could be attributed to lowering of TC.

We found that although the mean TG values at end line increased, the percentage of children within the reference range for TGs remained the same. Data on TG concentrations in Vitamin D supplemented children with T1D are scarce. A study was done by Javed *et al.* in obese adolescents where children received 100,000 IU Vitamin D3 orally once a month for 3 months, a significant increase in TG was noted [27]. In another study by Patwardhan *et al.*, one group with low 25 (OH) D concentrations was supplemented with 1000 IU Vitamin D daily for a period of 6 months while the other group received increased sunlight exposure. It was noted that while there was no change in TG in the Vitamin D supplemented group, authors found a non-significant increase in TG concentrations in group that received higher sunlight exposure [28].

With Vitamin D and calcium supplementation, we observed improvement in the bone profile and modest improvement in Vitamin D concentrations. Literature reports suggest that for higher increase in Vitamin D, higher doses up to 4000 IU/day may have to be used [29]. Marwah *et al.*, 2018, concluded that 1000 IU/day of Vitamin D might be required to achieve and maintain Vitamin D sufficiency in 97% of healthy adolescent girls, whereas 2000 IU/day would achieve sufficiency in 100% of girls [30]. Similar observations have been documented with a requirement of higher doses of 2000 IU daily in at-risk group children in the studies done by Sacheck *et al.* [31] and Lewis *et al.* [32].

We found an improvement in HbA1c concentrations, although the glycemic control was still out of the optimal range. The reason for poor glycemic control could be because most of the children in our clinic belong to underprivileged families and poor glycemic control in such families has been reported by Secrest *et al.*, 2011 [33]. In a study done by Aljabri *et al.*, 80 patients with T1D who had 25(OH) D concentrations <50 nmol/L were

Table 3: Regression analysis of factors associated with lipid profile improvement

Parameters	Biochemical predictors		Lifestyle predictors	
	Model I	Model II	Model III	Model IV
	Improvement in cholesterol category	Improvement in HbA1c category	Improvement in cholesterol category	Improvement in HbA1c category
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
HbA1C				
Cat1	0.6 (0.24–1.4)			
Cat2	0.7 (0.27–1.6)			
Cat3	1.5 (0.6–3.8)			
Cat4	1			
Improvement in cholesterol				
Cat1		0.43 (0.18–1.06)* (0.06)		
Cat2		0.57 (0.23–1.41)		
Cat3		0.38 (0.16–0.91)*		
Cat4		1		
Improvement in Vitamin D				
Cat1	0.3 (0.15–0.94)*	0.87 (0.36–2.13)		
Cat2	0.4 (0.16–0.97)*	0.97 (0.40–2.36)		
Cat3	0.38 (0.15–0.94)*	2.11 (0.88–5.03)		
Cat4	1	1		
Disease duration	0.44 (0.87–1.1)	0.67 (0.79–1.02)	0.95 (0.86–1.06)	1.03 (0.94–1.1)
Lifestyle				
Moderate activity			0.99 (0.99–1)	1.001 (1–1.002)
Sunlight			0.99 (0.98–1)	1.002(0.99–1.013)
Carbohydrate density				
Cat1			6.2 (0.88–44.26)* (0.06)	0.31 (0.048–2.02)
Cat2			5.4 (1.03–28.1)*	0.73 (0.14–3.76)
Cat3			5.99 (1.55–23.04)**	0.53 (0.13–2.15)
Cat4			1	1
Fat density				
Cat1			2.03 (0.31–13)	0.25 (0.03–1.66)
Cat2			1.09 (0.27–4.38)	0.40 (0.10–1.53)
Cat3			1.38 (0.4–4.75)	0.27 (0.09–0.83)
Cat4			1	1
Zn density				
Cat1			1.84 (0.7–4.86)	0.895 (0.36–2.22)
Cat2			1.64 (0.61–4.35)	0.479 (0.17–1.28)
Cat3			1.31 (0.54–3.2)	0.85 (0.34–2.11)
Cat4			1	1

\* $p < 0.05$ , \*\*  $p < 0.001$  OR: Odds ratio, CI: Confidence interval

supplemented with Vitamin D3 and improvement in glycemic control was observed in Vitamin D-replete patients [34]. Furthermore, a recent Egyptian study found that 3 months of supplementation with Vitamin D resulted in an improvement in glycemic control [35]. An Indian study by Sharma *et al.* reported a falling trend in HbA1c on supplementation with Vitamin D and calcium, though the difference was not statistically significant [36]. We did not find that the HbA1c was influenced by dietary intake and physical activity.

Our study has several limitations, an important one being we did not have a control group for this study; many of our children with diabetes were Vitamin D deficient; thus, having a control group without supplementation was not possible. However, at our center, we do annual tests on all children with diabetes who regularly attend clinics and have monthly follow-ups. In children

who were not supplemented with vitamin D with disease duration was  $>2$  years, mean lipid profile [3.22 mmol/L (baseline)–4.1 mmol/L (after 1 year)], Vitamin D [40 nmol/L (baseline)–44 nmol/L (after 1 year)], and HbA1c [9.1% (baseline) vs. 8.8 % (endline)] were not different at two successive annual checks, unlike in our study participants who had an improvement in lipid profile and glycemic control at end line. Another of our limitation was that the study participants' glycemic control was poor and remained so after supplementation. Strategies to improve the lipid profile in these children are of utmost importance.

## CONCLUSION

Daily supplementation with Vitamin D along with calcium in children with T1D resulted in an improvement in lipid profile and



also in glycaemic control. It may be a useful strategy for reducing their cardiometabolic risk.

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