

## Assessment of Vitamin D status in Type 1 Diabetes Mellitus in pediatric age group

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**Abstract**

**Background:** Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. Type-1-Diabetes Mellitus (T1DM) results from autoimmune destruction of beta cells leading to insulin deficiency. Vitamin D deficiency (VDD) may have a role in the pathogenesis and development of T1DM by regulating immune mechanism.

**Materials and Methods:** The aim of this study is to assess and evaluate the connection between fasting blood sugar (FBS), post prandial blood sugar (PPBS), glycated hemoglobin (HbA1c) and Vitamin D levels in children with T1DM. This study included 50 healthy controls and 50 previously diagnosed T1DM cases of both sex in the age group of 6-15 years. 25 hydroxy Vitamin D3 (25-OH Vit D3) level was estimated by Enzyme Linked Fluorescent Assay (ELFA), FBS, PPBS by GOD-POD method, HbA1c levels by Ion Exchange Resin method. Results: Results showed a decreased 25-OH Vit D3 and an increased FBS, PPBS and HbA1c levels which are highly significant ( $p < 0.001$ ) in T1DM cases than healthy controls. A highly significant negative correlation was observed between FBS, PPBS, HbA1c and 25-OH Vit D3 ( $P < 0.001$ ) in T1DM cases.

**Conclusion:** VDD has consistently been shown to be prevalent in children with T1DM, which plays an important role in its pathogenesis. Vitamin D, an immunomodulator is an important factor in glycemic control with subsequent prevention of T1DM and its further complications.

**Keywords:** Type 1 Diabetes Mellitus (T1DM); Vitamin D deficiency (VDD); 25 Hydroxy Vitamin D3 (25-OH D3); Fasting blood sugar (FBS); Post-prandial blood sugar (PPBS); Glycated hemoglobin (HbA1c).

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**Introduction**

Diabetes mellitus (DM) is a metabolic disorder resulting either from deficiency of insulin or resistance to its action causing increased blood glucose levels (hyperglycemia) which leads to several systemic complications. It is divided into two major forms. Those caused by pancreatic beta cell damage leading to deficiency of insulin secretion (T1DM) and those with insulin resistance at the level of skeletal muscle, liver and adipose tissue (Type-2 DM)<sup>[1]</sup>.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which the pancreas is unable to respond to stimulation with appropriate insulin secretion. Hyperglycemia develops when more than 70-90% of the insulin-producing beta cells are damaged. An autoimmune destructive process which plays a central role in the development of T1DM is facilitated by genetic susceptibility and by non-genetic factors like viral infections, toxic chemicals, deficiency of vitamin D and others. Vitamin D deficiency (VDD) is a non-genetic factor that appears to be associated with an increased risk of developing T1DM<sup>[2]</sup>. Incidence rates

of Type 1 diabetes mellitus vary widely by country, that as high as 30-40 cases per 1, 00,000 children in Finland and as low as 1 in 1, 00,000 in Japan and China<sup>[3]</sup>. There is very little data from India, but a study from madras suggests that diabetes in Indian children is present in a frequency of 10.5 per 1, 00,000 patient years. Prevalence of T1DM in Indian urban population is 0.26 per 1000<sup>[4]</sup>.

Vitamin D is a fat soluble vitamin. It resembles sterols in structure and functions like a hormone. There are three major sources for getting Vit D. Most people achieve their vitamin D needs through direct ultraviolet B (UVB)-mediated cutaneous synthesis. It can be taken up from food (e.g. fatty fishes and their oils) and through Supplementations. Activation of vitamin D requires two hydroxylation steps, the enzyme 25 hydroxylase (liver) or CYP2R1 leading to 25-hydroxyvitamin D3 (25-OHD3) and the enzyme 1- $\alpha$  hydroxylase (kidney) or CYP27B1 leading to the 1 $\alpha$ ,25-dihydroxyvitamin D3 [1 $\alpha$ ,25(OH)2D3], an active hormone<sup>[5]</sup>. Vitamin D and its metabolites are transported in the circulation by vitamin D-binding proteins megalin and cubilin<sup>[6]</sup>. Vitamin D exerts its actions in a variety of cell types by binding to the nuclear vitamin D receptor (VDR). The vitamin D status is usually assessed by measuring 25-hydroxyvitamin D3 (25-OH-D3) levels in the blood, a major circulating metabolite of vitamin D<sup>[7]</sup>. The VDR gene is on chromosome 12q12-14 spans nearly 100 kb. The CYP27B1 gene is also found on chromosome 12, at 12q.13.1-13.3, 10 Mb centromeric of the VDR gene. Mutations in CYP27B1 cause vitamin D-dependent

rickets and polymorphisms of the gene are associated with type I diabetes, Addison's disease, Graves' disease and Hashimoto's thyroiditis<sup>[8,9]</sup>. The CYP27B1 promoter (K1260) variant C is more often transmitted to offspring with type I diabetes.

Type-1 diabetes is characterised by almost total deficiency of insulin due to destruction of  $\beta$  cells of pancreas and immune system plays a central role in the destruction of the  $\beta$  cells. VDR is detected in almost all cells of the immune system, especially antigen-presenting cells like macrophages and dendritic cells and activated T cells which led to the investigation of a potential role for  $1\alpha,25(\text{OH})_2\text{D}_3$  as an immunomodulator in the prevention of T1DM<sup>[10,11]</sup>. Immune cells specially activated macrophages and dendritic cells not only have VDRs, but are also able to synthesize and secrete  $1\alpha,25(\text{OH})_2\text{D}_3$ <sup>[12]</sup>. As they possess the enzyme  $1\alpha$ -hydroxylase for the final activating step in the synthesis of  $1\alpha,25(\text{OH})_2\text{D}_3$ . Vitamin D inhibits the production of inflammatory interleukins like IL-12, IL-2, interferon  $\gamma$  and TNF- $\alpha$ . This may disrupt the production of Th1 cells, which are destructive for the pancreatic beta cells<sup>[13]</sup>.

Glycated hemoglobin (HbA1c) refers to the glucose derived products of normal adult hemoglobin. Glycation is a post-translation, non-enzymatic addition of sugar residue to amino acid of proteins. When there is hyperglycemia, proteins (haemoglobin) in the body undergo glycation. Among the glycated haemoglobins, the most abundant form is HbA1c. It remains inside the erythrocytes throughout its life span (120-days)<sup>[14]</sup>. Normal level of glycated hemoglobin (HbA1c) is about 4-7%. HbA1c level reveals mean glucose level over previous 8-10 weeks<sup>[14]</sup>. Many randomized, prospective clinical trials in type 1 diabetes have shown that achieving glycemic control significantly decrease the microvascular complications of diabetes. With 1% reduction in HbA1c levels associated with a 37% decrease in risk for microvascular complications and a 21% decrease in the risk of any end point or death related to diabetes.<sup>[15,16]</sup> Chronic diabetic complications can be divided into microvascular complications like retinopathy, neuropathy and nephropathy and macrovascular complications include cardiovascular, cerebrovascular and peripheral vascular disease. Severe microvascular and macrovascular complications can lead to renal failure which is the most common cause of hemodialysis, blindness or lower extremity amputations.<sup>[17]</sup> The aim of our study was to evaluate vitamin D status in children with T1DM and to correlate it with different clinical and laboratory parameters.

## Materials and Methods

This is the Prospective research design with randomized selection of children, which comprised of 100 study participants of age group 6-15 years of which

50 healthy controls (group A) and 50 already diagnosed T1DM cases (group B) based on standard American Diabetes Association (ADA) criteria. Both fasting and post prandial blood samples are collected with aseptic precautions. Samples for glucose and HbA1c estimation were collected in sodium fluoride and di-potassium EDTA tubes. After overnight fasting, about 4 ml of venous blood was drawn for analysis of FBS by GOD POD method in Biosystems Auto Analyzer, HbA1c by Ion Exchange Resin method using Erba Mannheim Semi autoanalyzer<sup>[18,19]</sup>. Serum was then subjected to estimate Vitamin D level by Enzyme Linked Fluorescent Assay (ELFA) in Mini Vidas immunoassay analyzer<sup>[20]</sup>. Then 1 ml of sample was collected after 2 hours of breakfast to estimate PPBS in all the 100 children. Children with T1DM attending the Out Patient and admitted in the Department of Paediatrics of Navodaya Medical College Hospital & Research Centre, Raichur were included in the study.

For the assessment of HbA1c for glycemic control, the participants were divided into three groups, that include good glycemic control (HbA1c  $\leq$  7%), moderate glycemic control (HbA1c 7.1 > 9%) and poor glycemic control (HbA1c > 9%).

The study divided the participants into three groups based on vitamin D levels according to National Health and Nutrition Examination Surveys 2003-2006. Participants having the level of vitamin D <12 ng/ml were included in group 1 (severely deficient), 12.1 - 20 ng/ml in group 2 (vitamin D deficient) and 20.1 - 30ng/ml in group 3 (insufficient). Patients who had the level of vitamin D  $\geq$  30 ng/ml were included in group 4 (sufficient)<sup>[21]</sup>. All of the children with T1DM were receiving insulin therapy during the investigation. Children <6 year and >15 years of age, Vitamin D supplementation, nutritional rickets, malnutrition, liver disease and end stage renal disease were excluded from this study. After the study was approved by institutional Ethical committee, informed written consent was taken from each participant of the study. The study was carried out in the department of Biochemistry, Navodaya Medical College from July 2015 to January 2016.

## Statistical analysis

SPPS 19.0 software version was used for performing statistical analysis. The results for different profiles were expressed as Mean $\pm$ standard deviation (SD). The statistical analysis was done by using student (unpaired) t-test, ANOVA(One way analysis of variance, and Pearson's correlation coefficient was used to evaluate the relationship between FBS, PPBS, HbA1c levels and  $25\text{OHD}_3$  levels. The p-value of <0.05 and <0.001 was considered significant & highly significant respectively.

## Results

Table 1: Characteristics of studied groups

Parameter		Control s	T1DM Cases		
			Severe Vitamin D deficiency	Vitamin D deficiency	Vitamin D insufficiency
Number		50, def=8(16%) insuff=9(18%) suff=33(66%)	6 (12%)	33 (66%)	11 (22%)
Age (6-15 years)	6-10 years	24 (48%)	1 (2%)	17 (34%)	6 (12%)
	11-15 years	26 (52%)	5 (10%)	16 (32%)	5 (10%)
Gender	Males	24 (48%)	1 (2%)	14 (28%)	8 (16%)
	Females	26 (52%)	5 (10%)	19 (38%)	3 (6%)
Duration of T1DM	< 5 years	-			
	> 5 years				
HbA1c (%) (n) & %	Good control (<7%)	50	-	-	6-10yr=03 (6%)
	Moderate control (7.1-9%)	-	-	11(22%) 6-10yr=9(18%) 11-15yr=2(4%)	08(16%) 6-10yr=3(6%) 11-15yr=5(10%)
	Poor control (>9%)	-	11-15yr=5(10%)	22(44%) 6-10yr=9(18%) 11-15yr=14(28%)	-

Table 2: Comparison of mean levels of vitamin D, FBS, PPBS and HbA1c in controls and T1DM cases

Parameter (mean± SD)	Controls (Group A)	T1DM cases (Group B)	p-value (Unpaired 't')
Age in years	10.08±2.83	11.02±2.22	0.067(70.05) NS,
Vitamin D	34.16±10.22	16.51±5.15	0.0001(<0.0001)*
FBS	84.42±11.04	178.68±42.92	0.0001(<0.0001)*
PPBS	121.88±6.66	250.22±57.86	0.0001(<0.0001)*
HbA1c	5.43±0.42	9.46±1.46	0.0001(<0.0001)*

\*highly significant

Table 2 shows a highly significant decrease in 25-OH D3 levels and a highly significant increase in FBS, PPBS and HbA1c levels in cases when compared to controls ( $p<0.001$ ).

Table 3: Comparison of vitamin D levels in diabetic children with laboratory parameters

Parameter (mean± SD)	Severe Deficiency <12 ng/ml	Deficiency 12.1 - 20 ng/ml	Insufficiency 20.1 - 30 ng/ml	p-value (one way ANOVA)
Age in years	9.00	8.88±1.11	9.50±0.84	0.47(>0.05) NS
11-15	13.80±1.10	12.81±1.05	12.0±1.22	0.49(<0.05) S
Duration of T1DM	5.50±2.17	3.97±2.05	3.45±1.69	0.134(>0.05) NS
FBS	251.50±14.80	181.36±29.71	130.91±19.40	0.0001(<0.0001)*
PPBS	343.17±24.43	256.79±37.92	179.82±26.96	0.0001(<0.0001)*
HbA1c	12.37±0.42	9.61±1.007	7.44±0.60	0.0001(<0.0001)*

\*highly significant

Table 3 shows that severely vitamin D deficient T1DM patients had a highly significant elevated levels of FBS, PPBS and HbA1c followed by patients with vitamin D deficiency than patients with insufficient vitamin D levels ( $p<0.001$ ).

**Table 4: Correlation of FBS, PPBS, HbA1c and Vitamin D levels in children with T1DM cases**

Parameter	25-OH D3	
	Pearson's correlation	p-value
FBS (mg/dl)	-0.872	0.0001(<0.0001)*
PPBS (mg/dl)	-0.924	0.0001(<0.0001)*
HbA1c (%)	-0.931	0.0001(<0.0001)*

\* Highly significant

Table 4 shows a highly significant negative correlation between FBS, PPBS, HbA1c and 25-OH D3 in T1DM cases ( $p < 0.001$ ).

## Discussion

According to Table 1, 22% of T1DM patients were 25-hydroxyvit D3 insufficient, 66% were deficient and 12% were severely deficient. 18% of controls were 25-hydroxyvit D3 insufficient, 16% were deficient and 66% had sufficient levels, which indicates that there is higher prevalence of 25-OH D3 deficiency in T1DM patients compared to controls, which is in agreement with Borkar et al and Branco et al<sup>[22,23]</sup>. In the present study, 25-OH D3 levels were highly significantly decreased ( $p < 0.001$ ) and FBS, PPBS and HbA1c levels were highly significantly increased ( $p < 0.001$ ) in T1DM children as compared to controls. Our study is in agreement with Soliman et al<sup>[24]</sup>. Vitamin D deficiency is common due to several factors such as reduced cutaneous synthesis (due to religious practices, seasonal variation, fear of cancer, and practice of not taking the child out, increase in pigmentation, increased indoor lifestyles, use of sunscreens), decreased dietary intake, air pollution, increasing rate of exclusive breast feeding and low maternal vitamin D. Concerning 25-hydroxyvitamin D3, group B (cases) had a highly significant lower serum levels than group A (controls) with  $P < 0.001$ . This could be due to beta islet cells destruction promoted by VDD in humans. This shows a strong connection between VDD and the incidence of T1DM, which explains low bone density in T1DM<sup>[25]</sup>. VDD leads to the increased levels of glucose and glycated hemoglobin<sup>[26]</sup>. It has been estimated that there is a strong connection between vitamin D deficiency, recurrence of symptoms of diabetes and the complications of T1DM. It is therefore suggested that the vitamin D levels of diabetic patients should be assessed on regular basis<sup>[27]</sup>.

Vitamin D has direct effect on B-islet cells, including improving insulin secretion and enhancing expression of vitamin D receptors<sup>[28]</sup>. Littorin et al. found that 25-hydroxyvitamin D3 was lower in patients with T1DM compared with controls whether they are newly diagnosed or after years of diagnosis<sup>[29]</sup>. This finding may support the idea that vitamin D deficiency may be an important factor behind the development of T1DM due to immunological background<sup>[30]</sup>. One study in Florida, a solar rich region in the United States found no difference in 25-OH D3 levels in diabetics compared to controls<sup>[31]</sup>. Our study showed insignificant difference between controls & cases with reference to age group.

Severely vitamin D deficient T1DM patients had a highly significant elevated levels of FBS, PPBS and HbA1c followed by patients with vitamin D deficiency than patients with insufficient vitamin D levels ( $p < 0.001$ ). We found a highly significant negative correlation between FBS, PPBS, HbA1c and 25-hydroxyvitamin D3 ( $p < 0.001$ ). Our study is in agreement with Soliman et al<sup>[24]</sup>. This shows that there is a close relationship between reduced serum 25-OH D levels and improper metabolic control among diabetics.

Vitamin D supplementation during pregnancy decreased the risk of the development of type 1 diabetes mellitus for newborns<sup>[32]</sup>. Destruction of beta cells begins in infancy or childhood and continues until T1DM is diagnosed. Supplementation of vitamin D at an early age also decreases the risk for developing T1DM<sup>[33]</sup>. Even after the onset of diabetes, it may improve glycemic control.<sup>[34]</sup> Supplementation of Vit D in pregnancy, lactation and early childhood protects against or reduces the severity of pancreatic insulinitis via a dual action on the pancreatic beta cells and the immune cells.<sup>[35,36]</sup>

## Conclusion

Vitamin D deficiency is associated with and suggests a role in the pathogenesis of T1DM. It acts as an immunomodulator, which has a good protection against pancreatic insulinitis. Supplementation of Vitamin D during pregnancy and early childhood decreases the risk of T1DM and improves glycemic control. We recommend creating awareness to increase sunlight exposure and intake of vitamin D rich food at community level and Vitamin D food fortification program at government level.

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