RESEARCH ARTICLE

Assessment of serum vitamin D level in children with type 1 diabetes mellitus: A cross-sectional study

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Abstract

Objectives: To estimate vitamin D levels in children with type 1 diabetes, and to evaluate its role in the pathogenesis and progress of the disease.

Method: The cross-sectional study was conducted at the Paediatric Department of Kafrelsheikh University Hospital, Egypt, from November 2019 to August 2021, and comprised children of either gender aged 3-18 years who were either inpatients or visiting the paediatric outpatient clinic. The subjects were enrolled into 3 groups. Those with newly diagnosed type 1 diabetes were in group A, those with established type 1 diabetes were in group B, and healthy children matched for age and gender and randomly selected were in the control group C. Glycated haemoglobin, serum fasting C-peptide, and serum vitamin D levels were evaluated using quantitative colorimetric determination, an automated analyser, and enzyme-linked immunosorbent assay, respectively. Data was analysed using SPSS 25.

Results: Of the 80 subjects, 30(37.5%) were in group A; 17(56.7%) boys and 13(43.3%) girls with mean age 7.77±2.95 years. In group B, there were 30(37.5%) subjects; 14(46.7%) boys and 16(53.3%) girls with mean age 9.6±3.62 years. There were 20(25%) subjects in group C; 10(50%) boys and as many girls with mean age 8.38±2.68 years ($p>0.05$). Glycated haemoglobin, serum fasting C-peptide and serum vitamin D was significantly different between the control group and the treatment groups ($p<0.05$). Between the treatment groups, group B had better markers than group A ($p<0.05$).

Conclusion: Serum vitamin D deficiency may play a role in the pathogenesis and insulin sensitivity in cases of type 1 diabetes.

Keywords: Diabetes mellitus, C-Peptide, Glycated haemoglobin, Colorimetry. **DOI: 10.47391/JPMA.EGY-S4-61**

Introduction

Type 1 diabetes mellitus (T1DM) represents a chronic autoimmune disease characterised by the degeneration of beta (β) cells of the pancreas which causes an inability to produce sufficient insulin.[1]

T1DM might be caused by the immune system destroying insulin-producing pancreatic β cells. Auto-reactive lymphocyte activation and cytokines' release induce apoptosis of pancreatic β cells which might play a significant role in the pathogenesis of T1DM.[2]

Vitamin D, which is a fat-soluble vitamin, is important not only in bone metabolism, but also as a transcription factor that promotes the secretion of insulin from β cells of the pancreas via vitamin D receptors (VDRs), which represent nuclear hormone receptors.[3]

Vitamin D is important in the modulation of the immune system, which in turn influences the onset of T1DM. Vitamin D is a powerful immune modulator that regulates the proliferation and differentiation of cells, activation of lymphocytes, and production of cytokines.[4] Furthermore, it reduces the expression of Fas and major histocompatibility complex (MHC) class I molecules, inhibiting pancreatic β cell apoptosis.[5]

The current study was planned to investigate vitamin D levels in T1DM children, and to evaluate its role in the pathogenesis and progress of the disease.

Patients and Methods

The cross-sectional study was conducted at the Paediatric Department of Kafrelsheikh University Hospital, Egypt, from November 2019 to August 2021. After approval from the institutional ethics review committee, the sample was raised from among children of either gender aged 3-18 years who were either inpatients or visiting the paediatric outpatient clinic using the convenient sampling technique. Children with T1DM were diagnosed by clinical and laboratory findings, according to the American Diabetes Association (ADA) diagnostic criteria.[6] Children who had bone diseases, liver diseases, kidney diseases, autoimmune diseases, or melanoma, or those receiving anticonvulsant drugs were excluded. The subjects were enrolled into 2 groups. Those with newly diagnosed T1DM were in group

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A, and those with established T1DM were in group B. Besides, healthy children matched for age and gender were randomly selected and acted as control group C.

After taking informed consent from the parents or guardians of all the subjects, data was collected regarding medical history, personal history, perinatal history, developmental history, nutritional history, present history, and past history. This was followed by a thorough clinical examination, and pathological tests, including complete blood count (CBC), platelet, and total leukocytic count (TLC), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum creatinine and serum fasting blood glucose (FBG). Glycated haemoglobin (HbA1c) level was measured using quantitative colorimetric determination (ADAMS A1C HA-8180T, Arkray, Europe). Fasting C-peptide level was measured using an automated analyser (Cobas e-411, Roche, Switzerland), and serum level of vitamin D was measured using Biokit enzyme-linked immunosorbent assay (ELISA) (25 OH D ELISA kit, MyBioSource, United States).

From each subject, 7mL venous blood was drawn aseptically via venipuncture; 2mL was injected into an ethylenediaminetetraacetic acid (EDTA) vacutainer tube for CBC and HbA1c, another 2mL was injected into a plain vacutainer tube for AST, ALT, creatinine and FBG, and the remaining 3mL was injected into a sterile plain vacutainer tube, allowed to clot at a 37°C for about 10min, and centrifuged at 3000rpm for 10min.

Data was analysed using SPSS 25. Means and standard deviations or medians and interquartile range (IQR) were used to present quantitative variables. Frequencies and percentages were used to present categorical variables. Chi-square test and Mann-Whitney test (U), one-way analysis of variance (ANOVA) (F) and Kruskal Wallis test were used, as appropriate. Fisher least significant difference (LSD) test and pairwise comparisons were used to distinguish at least one group from the others. Pearson and Spearman rank correlation coefficients were used to evaluate the strength and direction of a linear relationship between two continuous variables. To determine the extent to which a dependent variable and one or more independent variables had a linear relationship, linear stepwise regression analysis was used.[7] P<0.05 was considered statistically significant.

Results

Of the 80 subjects, 30(37.5%) were in group A; 17(56.7%) boys and 13(43.3%) girls with mean age 7.77±2.95 years. In group B, there were 30(37.5%) subjects; 14(46.7%) boys and 16(53.3%) girls with mean age 9.6±3.62 years. There were 20(25%) subjects in group C; 10(50%) boys and as many

Table-1: Baseline characteristics and laboratory investigations.

Parameters	Groups Newly diagnosed DME n=30 (%)	stablished DM group n=30 (%)	Control group n=20 (%)	Test F	*p-value*
Gender:					
Female	17 (56.7%)	14 (46.7%)	10 (50%)	$\chi^2$0.617	0.735
Male	13 (43.3%)	16 (53.3%)	10 (50%)		
Mean Age (years)	7.77 ± 2.95	9.6 ± 3.62	8.38 ± 2.68	2.59	0.082
Mean Haemoglobin (g/dl)	12.52 ± 0.85	12.13 ± 0.72	12.09 ± 0.7	1.807	0.171
Mean TLC (10^9/L)	5.51 ± 0.99	5.73 ± 0.88	5.33 ± 0.76	0.656	0.522
Mean Platelet (10^3/µl)	270.3 ± 37.57	284.6 ± 30.42	293.65 ± 60.06	1.978	0.145
Tukey HSD		P1=0.21 P2=0.482 P3=0.024*			
Mean Creatinine (mg/dL)	0.5 ± 0.13	0.49 ± 0.14	0.49 ± 0.16	0.034	0.966
Mean ALT (U/L)	16.7 ± 3.37	16.4 ± 2.91	17.4 ± 2.01	0.722	0.489
Mean AST (U/L)	19.2 ± 3.82	17.87 ± 3.76	18.5 ± 2.63	1.066	0.499

χ^2: Chi-square test, DM: Diabetes mellitus, TLC: Total leukocytic count, ALT: Alanine transferase, AST: Aspartate transferase, HSD: Honestly significant difference, F: One-way analysis f variance (ANOVA) test. *P value is significant. P1: Difference between groups A and B. P2: Difference between groups B and C. P3: Difference between groups A and C,

Table-2: Glycaemic profile and serum vitamin D levels.

Parameters	Groups Newly diagnosed DME n=30 (%)	stablished DM group n=30 (%)	Control group n=20 (%)	Test F	*p-value*
Mean HbA1c (%)	10.29 ± 1.17	8.39 ± 0.35	4.78 ± 0.24	317.891	<0.001*
Mean Fasting blood glucose (mg/dl)	249.93 ± 26.28	143.83 ± 8.6	95.0 ± 7.23	539.301	<0.001*
Tukey HSD		**P1<0.001* P2<0.001* P3<0.001***			
Median Fasting C-peptide (IQR)	0.4 (0.3 – 0.4)	0.35 (0.2 – 0.43)	2.9 (2.45 – 3.15)	46.446	<0.001*
Pairwise		**P1>0.05 P2<0.001** P3<0.001***			
Hospital admission for Diabetic KA, N of times	Diabetic Ketoacidosis	1.5 (1 – 3)		-	-
Median (IQR)	3.5 (3 – 4.25)				
Level:					
Deficiency	27 (90%)	7 (23.3%)	0 (0%)	χ^2	
Suboptimal	3 (10%)	23 (76.7%)	0 (0%)	0.617	0.735
Normal	0 (0%)	0 (0%)	20 (100%)		
Mean Vitamin D (ng/mL)	15.43 ± 3.99	22.27 ± 2.77	47.65 ± 7.3	298.27	<0.001*
Tukey HSD		**P1<0.001* P2<0.001* P3<0.001***			

χ^2: Chi-square test, HbA1c: Glycated haemoglobin, DM: Diabetes mellitus, HSD: Honestly significant difference, IQR: Interquartile range, KA: Ketoacidosis, F: One-way analysis of variance (ANOVA) test. KW: Kruskal Wallis test. *P value is highly significant.
P1: Difference between groups A and B; P2: Difference between groups B and C; P3: Difference between groups A and C.

Table-3: Correlation between vitamin D and study variables.

	r-value	*p-value*
Age (year)	0.06	0.647
Gender	-0.035	0.792
Disease duration (days)	0.664	<0.001*
Hospital admission	-0.546	<0.001*
Haemoglobin (g/dl)	0.168	0.199
TLC (10^9/L)	0.023	0.863
Platelet count (10^3/µl)	0.095	0.469
Creatinine (mg/dL)	-0.005	0.971
ALT (U/L)	-0.152	0.246
AST (U/L)	0.144	0.271
HbA1c (%)	-0.865	<0.001*
Fasting blood glucose (mg/dl)	-0.725	<0.001*
Fasting C peptide	-0.055	0.677

r: Spearman rank correlation coefficient, TLC: Total leukocytic count, ALT: Alanine transferase, AST: Aspartate transferase, HbA1c: Glycated haemoglobin. *P value is highly significant.

girls with mean age 8.38±2.68 years (p>0.05) (Table 1).

HbA1c, serum fasting C-peptide and serum vitamin D levels were significantly different between the control group and the diabetic groups (p<0.05). Between the two diabetic groups, group B had better markers than group A (p<0.05) (Table 2).

A strong positive correlation between serum vitamin D and T1DM duration was found, while there was a strong negative correlation between serum vitamin D and hospital admission, FBG and HbA1c (Table 3).

Discussion

T1DM prevalence estimates can be used to investigate its diverse aetiology, as well as the role of inheritance and environment in its development. Furthermore, prevalence rates are critical for the organisation of national diabetes medical care.[8]

The median disease duration of DM in newly-diagnosed group A children was 5 days in the current study, while it was 15 months in the established group B. This was in line with an earlier study.[9] Another study[10] reported the median duration of T1DM children to be 17 months, ranging 3-52 months.

The current study reported a slight increase in Hb in newly-diagnosed children compared to other groups, but there was no significant difference among the groups (p=0.17). Possible explanations include a reaction to generalised hypoxia caused by vascular disease or a reaction to testosterone, which has been shown to be elevated in TqDM.[11] Aside from testosterone's erythropoietic effects, insulin-like growth factors-1 and -2 and insulin itself promote the production of erythropoietin in astrocytes.[12,13]

The current study reported higher HbA1c and FBG in diabetic children than in controls, which is in alignment with other studies.[10,14,15]

When the conditions are physiologically favourable, proteins are regularly glycated during numerous biochemical reactions. Glycation of Hb, on the other hand, occurs through a non-biochemical reaction between the N-terminal end of the chain and glucose, resulting in the formation of a Schiff base.[16] Amadori products are produced from the Schiff base during the rearrangement, the most well-known of which is HbA1c.[17]

The current study reported significantly lower fasting C-peptide level in diabetic children compared to the controls. C-peptide is a proinsulin component that is cleaved in equimolar amounts to endogenous insulin before co-secretion with insulin from beta cells of the pancreas. Moreover, in T1DM, there is no insulin or very little insulin production, and C-peptide has been reported to be very low.[18]

In the present study, vitamin D level was significantly lower in diabetic groups than the controls, which is similar to earlier results.[9]

Another study[19], reported that diabetic children had significantly lower serum levels of 25-hydroxy vitamin D (25-OHD) than controls. The Diabetes Incidence Study in Sweden (DISS) discovered that the levels of 25-OHD in new-diagnosed T1DM were lower than in controls (p=0.001). After an 8-year follow-up, vitamin D levels in T1DM patients were also low .[20]

This could be explained by the fact that in humans, vitamin D deficiency may promote beta cell destruction. Furthermore, the presence of T1DM is associated with low vitamin D concentration, whereas the decline in 25-OHD levels after diagnosis is a result of DM-related metabolism disruption. This could explain why bone density is lower in T1DM than in T2DM.[21] Another potential explanation is that vitamin D has direct effects on B-cells, such as improving insulin secretion, increasing VDR expression, and improving islet morphology.[22]

The current study found a strong positive correlation between serum vitamin D and disease duration, while there was a strong negative correlation between serum vitamin D and hospital admission, FBG and HbA1c.

A study[10] reported a positive correlation between serum vitamin D and disease duration. Soliman et al.[19] reported that there were significant strong negative correlations of 25-OHD with FBG and HbA1c. Bulum et al.[23] found HbA1c to be inversely related to serum vitamin D levels before and after supplementation with vitamin D.

In line with th current results, a study[24] identified high mean HbA1c and a significant inverse correlation between vitamin D and HbA1c. Wulandari et al.[25] stated a significant difference in HbA1c levels in T1DM compared to controls, and a significant negative correlation between HbA1c levels in T1DM and levels of vitamin D.

In contrast to the current findings, El Baba et al.[26] did not report any correlation between vitamin D and HbA1c levels among diabetic children. Moreover, they found no correlation between control with diabetes and vitamin D level variations in a population with T1DM in Lebanon. A study[27] reported that treatment with vitamin D3 caused better glycaemic control in patients with T1DM. Furthermore, vitamin D3 supplementation improved HbA1C in all glycaemic control groups, including poor, fair and good.

In the current study, there were several mechanisms that may explain vitamin D deficiency and poor glycaemic control. First, the patients spent most of the day indoors, with decreased outdoor activities and exercise, especially in the summer season, because of excessively hot weather. Subsequently, deterioration of glycaemic control occurs, as well as low exposure to the sun. Second, insulin resistance plays a large role in the T1DM disease process than is generally known.

Considering the effect of vitamin D on insulin release and effect, it appears useful to evaluate vitamin D levels in diabetic patients' serum and, if necessary, prescribe supplements.

Conclusion

Vitamin D levels in the blood may play a role in T1DM pathogenesis. Vitamin D supplementation should be regarded as a promising treatment for T1DM.

Limitation: The sample size was not calculated and the study was conducted on a small number of participants. This could influence the power of the study and the results could not be generalised.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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