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Vitamin D and Its Association with Glycemic Status in Bangladeshi Adults with Newly Detected Type 2 Diabetes Mellitus

Md. Firoj Hossain[1], Tahniyah Haq[2*], Md. Fariduddin[2], Shahjada Selim[2], M. A. Hasanat[2], Md. Shahed-Morshed[3]

[1]Department of Endocrinology, Mugda Medical College, Dhaka, Bangladesh
[2]Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[3]Emergency Medical Officer, Kurmitola General Hospital, Dhaka, Bangladesh
Email: dr.firojhossain@gmail.com, *tahniyah81@bsmmu.edu.com, *tahniyah@bsmmu.edu.bd, dr.md.fariduddin@gmail.com, selimshahjada@jmail.com, hasantdr@yahoo.com, shahedk62@gmail.com

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Abstract

Background: Very limited data are available regarding the association of vitamin D with glycemic status among adults with newly detected type 2 diabetes mellitus (T2DM) in Bangladesh. ***Objectives***: To determine vitamin D status and its association with glycemic status in Bangladeshi adults with newly detected T2DM. ***Materials and Methods***: This cross-sectional study was carried out in 102 newly detected T2DM diagnosed on the basis of the American Diabetes Association 2017 criteria (age: 42.95 ± 10.68 yrs.; m/f: 44/58) and equal number of age and sex matched controls (age: 40.43 ± 11.04 years) recruited consecutively from the Department of Endocrinology, BSMMU to measure serum vitamin D by high performance liquid chromatography method. ***Results***: Both vitamin D deficiency (<20 ng/ml) (87.3% vs. 74.5%, $p < 0.03$) and severe vitamin D deficiency (<10 ng/ml) (56.2% vs. 26.3%, $p < 0.001$) were significantly higher in people with T2DM than control population. The mean level of 25(OH)D was significantly lower in adults with T2DM than control population (12.41 ± 6.85 ng/ml vs. 15.74 ± 6.25 ng/ml, $p < 0.001$). A significant inverse correlation was observed between vitamin D & HbA_1c ($r = -0.249$, $p = 0.011$) in patients with T2DM. HbA_1c was linearly associated with vitamin D ($\beta = -0.26$, $p = 0.009$) and severe vitamin D deficiency by binary (OR = 1.37, $p = 0.003$) and multinomial logistic regression ($HbA_1c \geq 10\%$: OR = 4.25, $p = 0.04$) in people with T2DM after adjustment for age and BMI. **Conclusions:** Severe vitamin D deficiency was positively associated with T2DM and inversely associated with HbA_1c in patients with newly detected T2DM.

Keywords

Vitamin D, Type 2 Diabetes Mellitus, Vitamin D Deficiency, Glycated Hemoglobin A_1c

1. Introduction

Diabetes mellitus (DM) and vitamin D deficiency (VDD) are both disorders of high prevalence in the whole world. Various studies suggest that vitamin D deficiency may play a major role in the causation of many chronic diseases including DM [1] [2]. Evidence generated from prospective studies in European and American population showed a significant inverse association between vitamin D levels and risk for type 2 DM (T2DM) [3] [4]. VDD has been reported to be more common among south Asians with T2DM living in the UK compared with people free of DM [5]. Both β-cell function and/or insulin sensitivity can be affected by VDD. Some of the proposed mechanisms that are related to insulin secretion include expression of vitamin D receptors in the β-cells of pancreas, location of vitamin D response element in human insulin gene and role of vitamin D in maintenance of normal calcium homeostasis. On the other hand, presence of vitamin D receptors in skeletal muscle, role of cytokines in causing insulin resistance and improvement of insulin mediated glucose utilization with down regulation of cytokines production following vitamin D therapy support a role of vitamin D in insulin resistance [6] [7] [8]. However, there are paucity data on the association of vitamin D status with glycemic status in newly detected T2DM patients in the literature. Therefore, this study was undertaken to see vitamin D status in adults and its association with glycemic status in newly detected T2DM of Bangladeshi population.

2. Materials and Methods

This observational cross-sectional study was carried out in the Department of Endocrinology, BSMMU over a period of one year between March 2017 to March 2018.

Ethics: The study protocol was approved by Institutional Review Board, BSMMU (No. BSMMU 2017/4060). Informed written consent was taken from each participant.

Study design: In this study, 102 adults with newly detected T2DM and equal number of age and sex matched controls were included by consecutive purposive sampling. Patients who were currently taking or had received vitamin D or calcium within the last 120 days of sample collection; or those with known liver disease, renal disease, severe heart failure, autoimmune disease, metabolic bone disorder, malabsorption syndrome, active malignancy, concurrent critical illness; or pregnancy and lactation were excluded from the study. Data were collected using pretested semi-structured questionnaires. Participants were asked

about their socio-demographic statuses and factors affecting vitamin D level. Height, weight, waist circumference and blood pressure of each participant were measured as per standard procedures.

Biochemical analysis: About 10 ml of venous blood was collected in sample tubes covered by aluminum foil from each participants after overnight 8 to 10 hours fasting. After 10 - 15 minutes of collection, blood sample tubes were placed in a centrifuge and spun at 3000 rpm for 10 minutes in a dark room to obtain serum. Serum was stored appropriately at −20˚C and was analyzed for serum HbA_1c and 25 hydroxyvitamin D {25(OH)D} within a week of sample collection. Vitamin D was measured using an automated analyzer by HPLC (high performance liquid chromatography) for the quantitative determination of 25(OH)D in human plasma by HPLC 25-OH-D assay (WAFFEN 029) in Centre for Advanced Research in Sciences, University of Dhaka. HbA_1c was measured using the NGSP certified Bio-Rad D-10™ HbA_1c Program 220-0101, USA. Plasma glucose was measured by enzymatic colorimetric test using glucose oxidase method. Serum 25(OH)D was measured by 20 series prominence HPLC analyzer with a coefficient of variability (CV) 2.6% - 4.9%. The method used here could detect serum 25(OH)D values from 5 to 100 ng/ml.

Operational definitions: Newly detected T2DM was defined as patient fulfilling ADA (American Diabetes Association) 2017 criteria {fasting blood glucose ≥ 7 mmol/L, 2 hours plasma glucose after 75 gm OGTT (Oral glucose tolerance test) ≥ 11.1 mmol/L (IGT), HbA_1c ≥ 6.5% or in a patient with classical symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 11.1 mmol/L} for the first time at presentation without any history of ketoacidosis and clinical features suggesting other types of DM [9].

Vitamin D status was defined by Endocrine Society clinical practice guideline, 2011 into vitamin D sufficiency, insufficiency and deficiency with the cut off value of 30, 20 - 29.9 & <20 ng/ml respectively [10]. Vitamin D deficiency was further categorized as mild to moderate (10 - 19.9 mg/ml) and severe deficiency (<10 ng/ml) [11].

Sample size calculation: The minimum sample size calculated was 60 using the formula, $n = (Z^2 \times p \times q) \div d^2$ for cross sectional study. The prevalence of vitamin D deficiency in newly detected type 2 diabetes mellitus (p) was 81% [12]. 95% confidence interval (z) and 10% margin of error (d) were used. As facility permitted we enrolled 102 subjects.

Statistical analysis: Data were analyzed using computer-based SPSS program (version 22.0). Analyzed data were described in frequencies (percentages) for qualitative value and mean (±SD) for quantitative values. Comparison of serum 25(OH)D level and frequency of vitamin D deficiency between newly detected T2DM and controls were done by Student's unpaired t-test and Chi-square test respectively. One-way ANOVA was used to compare vitamin D level among different levels of glycemia. Correlation was analyzed by Pearson's correlation test between serum 25(OH)D and plasma glucose (fasting and 2 hours after 75 gm OGTT), HbA_1c in patients with T2DM. Linear regression between HbA_1c

(independent) and 25(OH)D (dependent) was also done in people with T2DM. Binomial and multinomial logistic regression analysis were done to see whether HbA_1c (covariate) and its category (factor) were associated with development of severe VDD (dependent variable). Statistical significance was set at $p < 0.05$.

3. Results

A total of 102 adults with newly detected T2DM and equal number of controls (free of DM) were studied. Both cases and controls were comparable in age, gender, BMI and blood pressure. The mean age of the people with newly detected T2DM and controls were (42.95 ± 10.68 vs. 40.43 ± 11.04 years; $p = 0.10$) and BMI (26.33 ± 4.30 vs. 25.52 ± 4.38 kg/m^2; $p = 0.19$). Most of the participants came from urban area. Significant differences were seen in area of residence, occupation, level of education and central obesity. Most of the confounding factors for vitamin D level (smoking, sunlight exposure, physical activity and family income) were not significantly different between two groups (Table 1).

Table 1. Socio-demographic characteristics and personal history of the study participants (n = 204).

Variables	Diabetes (n = 102)	Control (n = 102)	*p*
	Frequency (%)		
Gender (female)	58 (56.9)	58 (56.9)	1.00
Urban resident	65 (63.7)	90 (88.2)	**0.001**
Occupation			
Housewife	51 (50.0)	31 (30.4)	
Service holder/businessman	41 (40.2)	54 (52.9)	**0.01**
Others*	10 (9.8)	17 (16.7)	
Highest educational status			
Primary	18 (17.6)	08 (7.8)	
Secondary	46 (45.1)	29 (28.4)	**0.001**
Higher secondary and above	38 (37.3)	65 (63.7)	
Monthly household income < 50 thousands taka	93 (91.2)	96 (94.1)	0.59
Current smokers	05 (4.9)	09 (8.8)	0.31
Physically active[¶]	50 (49.9)	50 (49.9	0.29
Adequate sunlight exposure time[†]	43 (42.2)	32 (31.4)	0.15
Adequate body surface area of sunlight exposure[¥]	14 (13.7)	18 (17.6)	0.70
Obese (BMI ≥ 25 kg/m^2)	59 (57.8)	52 (51.0)	0.55
Centrally obese (WC: male ≥ 90, female ≥ 80 cm)[£]	76 (74.5)	56 (54.9)	**0.003**
Hypertensive (BP ≥ 140/90 mm-Hg or on antihypertensive)	29 (28.4)	20 (19.6)	0.14

Within parenthesis are percentages over column total of each variable; Significance by chi-square test. *Others: unemployed (5), day laborer (4), retired (4), students (14); [¶]Walking for ≥150 min/week (at least 3 days a week); [†]at least 3 days a week ≥ 10 minutes [13]; [¥]Sunlight exposure of ≥20% of body surface area (face, arms, hands & legs); [£]Cut-off values for central obesity including waist circumference for male and female were ≥90 and ≥80 cm [14].

3.1. Vitamin D Status and T2DM

Although both groups had mean vitamin D level in VDD category, it was significantly lower in newly detected T2DM compared to control population (12.41 ± 6.85 vs. 15.74 ± 6.25 ng/ml; $p < 0.001$).

The frequency of vitamin D sufficiency, insufficiency and deficiency was present in 4.9%, 7.8% and 87.3% respectively in people with T2DM and 3.9%, 21.6% and 74.5% respectively in control population. Among the people with VDD, mild to moderate vitamin D deficiency (10 - 19.9 ng/ml) and severe vitamin D deficiency (<10 ng/ml) were present in 43.8% & 56.2% people with T2DM and 73.7% & 26.3% people with controls respectively. Both the associations were statistically significant (Figure 1).

3.2. Vitamin D and Glycemic Status

A decreasing trend of mean vitamin D level was seen with ascending category of HbA_1c without significant association (HbA_1c: <7% vs. 7% - 9.99% vs. ≥10%: 16.02 ± 9.42 ng/ml vs. 12.20 ± 6.04 ng/ml vs. 11.23 ± 6.60 ng/ml respectively; $p = 0.08$) in people with T2DM (Table 2).

Correlation showed an inverse relationship between serum 25(OH)D concentration and HbA_1c in newly detected T2DM patients ($r = -0.19$; $p = 0.05$) (Figure 2) but not with FPG ($r = 0.04$; $p = 0.71$) and plasma glucose 2 hours after 75 gm glucose load ($r = -0.9$; $p = 0.37$).

Linear regression analysis showed that, 1% increase of HbA_1c was associated with 0.26 ng/ml reduction of serum 25(OH)D level in T2DM population after adjustment for age and BMI (Table 3(a)).

Multinomial logistic regression analysis showed that, compared with HbA_1c < 7%, higher categories of HbA_1c of 7% - 9.99% and ≥10% were associated with 3.19 and 6.71 times increased risk of development of severe VDD respectively.

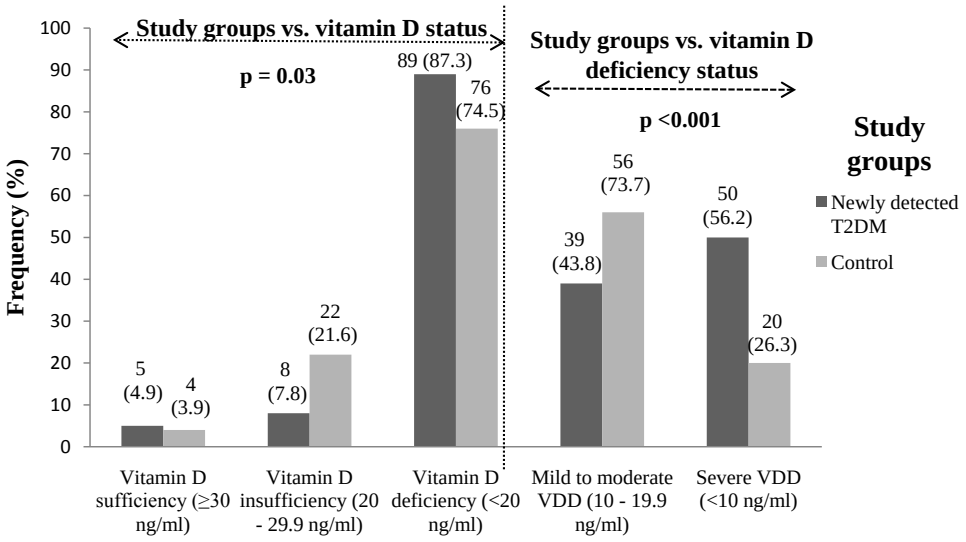

Figure 1. Vitamin D status in the study population (n = 204). Chi-square test was used to compare between groups; VDD = vitamin D deficiency.

Table 2. Vitamin D level at different levels of glycemia in newly detected T2DM (n = 102).

Vitamin D	HbA_1c (%)			*p*
	<7	7 - 9.9	≥10	
Serum 25(OH)D (ng/dl) [Mean ± SD]	16.02 ± 9.42	12.20 ± 6.04	11.23 ± 6.60	0.084

One-way ANOVA was done.

Table 3. (a) Linear regression analysis of 25(OH)D level as dependent variable and HbA_1c as independent variable in people with T2DM (n = 102); (b) Multinomial logistic regression analysis of severe vitamin D deficiency (<10 ng/ml) as dependent variable and HbA_1c category as independent factor in people with T2DM (n = 102).

(a)

	Unadjusted	Adjusted*
B (95% CI)	−0.76 (−1.37, −0.18)	−0.80 (−1.40, −0.20)
β	−0.25	−0.26
p	**0.01**	**0.009**

B = regression coefficient; CI = confidence interval, *adjusted for age and BMI.

(b)

HbA_1c category	Unadjusted		*p*	Adjusted*		*p*
	B	OR (95% CI)		B	OR (95% CI)	
<7%	1 (reference)			1 (reference)		
7% - 9.99%	1.16	3.19 (1.59, 6.40)	**0.001**	0.67	1.95 (0.54, 7.09)	0.31
≥10%	1.90	6.71 (2.90, 15.53)	**<0.001**	1.45	4.25 (1.08, 16.72)	**0.04**

B = regression coefficient; OR = odds ratio; CI = confidence interval *adjusted for age and BMI.

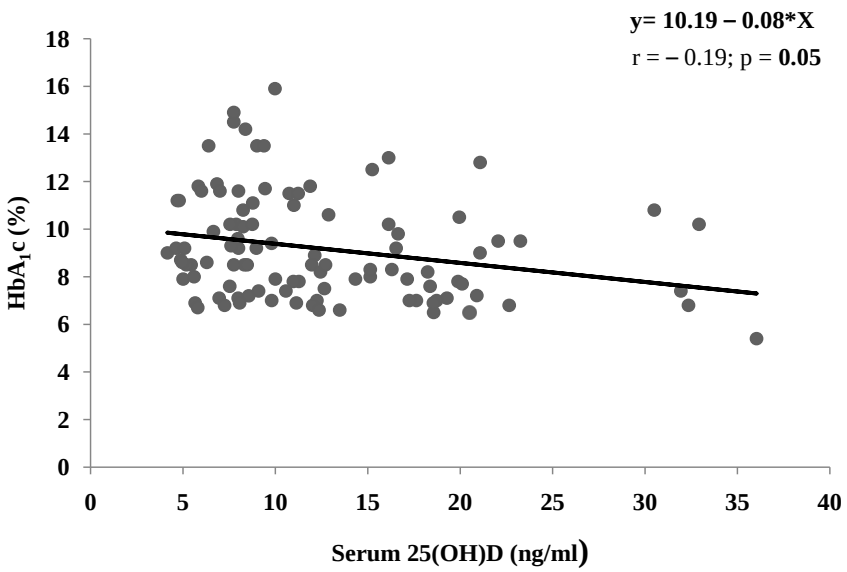

Figure 2. Correlation of vitamin D with HbA_1c in newly detected T2DM (n = 102). By Pearson's correlation test; r = Pearson's correlation coefficient.

However, after adjustment for age an BMI the significance persisted only with $HbA_1c \geq 10\%$ (**Table 3(b)**).

4. Discussion

In this study, we found that the mean serum 25(OH)D level was significantly lower in people with T2DM than the control population. Similarly, the frequency of VDD (<20 ng/ml) and severe VDD (<10 ng/ml) were significantly higher in participants with newly detected T2DM than the control population. Serum vitamin D level had a significant inverse correlation with HbA_1c in people with T2DM. HbA_1c was linearly associated with 25(OH)D in patients with T2DM. HbA_1c and its highest category ($HbA_1c \geq 10\%$) were associated with increased risk of severe VDD in people with T2DM.

In the present study, mean serum 25(OH)D was significantly lower in newly diagnosed T2DM compared to controls. Similar results were reported in different studies [15] [16] [17] [18]. The mean serum vitamin D concentration found to be in VDD and insufficiency group in previously conducted studies in Bangladesh [19] [20]. The mean serum vitamins D of these two studies were higher than our study. This may be due to a different method of vitamin D estimation. As, vitamin D is a steroid hormone and protein-bound, the immune-based assay (chemiluminescent assay, radioimmunoassay) may overestimate the 25(OH)D level due to simultaneous measurement of other circulating forms [21]. Besides history of sunlight exposure time and exposed body surface area were not mentioned in those studies. In our study, mean 25(OH)D in adults with T2DM was relatively lower than those published in Western studies [22] [23]. This might reflect the high prevalence of vitamin D deficiency in our normal population, which might be related to ethnicity or genetic predisposition, skin complexion, decreased sun exposure (due to clothing), low milk intake and lack of vitamin D fortification program. In addition, geographical location, occupation, level of education, socioeconomic status of the population may influence the frequency of vitamin D deficiency [24].

In our study, the people with T2DM had significantly higher percentages of VDD than people free of DM. Other studies conducted on different population found similar findings [16] [25]. However, a lower rate of VDD (2% - 30%) was reported in European adults with T2DM. Along with the previously mentioned causes, use of different cut off value (12 ng/ml) to define VDD might be an important cause of this different result [17]. Previous studies from Bangladesh reported lower prevalence of VDD (27.5% and 30% in people with T2DM [16] [17]. Again, this might be due to a different method of vitamin D estimation and different sample size.

There was an inverse relationship between serum 25(OH)D and HbA_1c in our study. Other studies' findings were consistent with our result [26] [27]. On the contrary, others found no negative correlation between vitamin D and HbA_1c [28] [29]. Similarly, vitamin D supplementation in people with T2DM showed mixed results on HbA_1c changes [30] [31]. As the possible action of vitamin D

on enhancing insulin secretion depends on reserved function of the pancreatic beta islet cells, the variability in responding to vitamin D supplementation in glycemic control could be due to the variability in the reserved beta islet cell function in T2DM [32].

We also found that HbA_1c and its highest category ($HbA_1c \geq 10\%$) were associated with severe VDD. One study also found association of VDD with HbA_1c status (≥7% vs. <7%) [33]. However, the direction of association between the vitamin D status and glycemic status in people with T2DM remains unresolved.

5. Conclusion

Vitamin D was lower in patients with T2DM than in control population, who also had a higher frequency of severe vitamin D deficiency. Vitamin D had significant inverse association with HbA_1c in people with T2DM.

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Conflicts of Interest

The authors declare that they have no conflict of interest concerning this article.

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