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Evaluation of vitamin D relationship with type 2 diabetes and systolic blood pressure

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ABSTRACT

Objective: To investigate whether relationships exist among vitamin D, type 2 diabetes mellitus (T2DM), and blood pressure in Trinidadian subjects with T2DM.

Research design and methods: This was a case–controlled study to determine if vitamin D levels were lower in patients with T2DM. After data analysis, an exploratory hypothesis of vitamin D relationship to systolic blood pressure (SBP) was developed. Plasma calcifediol (25(OH)D) concentrations were used as a measurement for vitamin D levels and were determined by ELISA. Cholesterol levels were measured by an automated dry chemistry analyzer and blood pressure was measured using an automatic blood pressure monitor.

Results: There was no significant difference (p=0.139, n=76) in 25(OH)D levels between patients with T2DM and controls. Subjects with SBP above 130 mm Hg were 8 times more likely to have a 25(OH)D plasma concentration above 25 ng/mL (OR 7.9 (2.2 to 28.7)), and were 5 times (OR 4.7 (1.7 to 15.1)) more likely to have a 25(OH)D plasma concentration above 30 ng/mL (OR 7.5 (2.3–24.2)). Vitamin D levels moderately and positively correlated with SBP (r_s=0.38, p=0.001).

Conclusions: There was no significant difference in the 25(OH)D levels between patients with T2DM and controls (p=0.139). Patients with SBP under 130 mm Hg were 8 times more likely to have a vitamin D level above 25 ng/mL (OR 7.9 (2.2 to 28.7)). Further investigations are required to examine the relationship between vitamin D and SBP.

Significance of this study

What is already known about this subject?

- An unclear relationship between type 2 diabetes mellitus (T2DM) and the vitamin D axis.
- Vitamin D levels are lower in hypertensive individuals as compared with normotensive individuals.

What are the new findings?

- Vitamin D levels are higher in patients with systolic blood pressure (SBP) above 130 mm Hg as compared with patients with SBP lower than 130 mm Hg.

How might these results change the focus of research or clinical practice?

- Future studies of vitamin D relationship to blood pressure and T2DM need to be conducted in tropical regions since vitamin D is regarded as a 'sunshine vitamin'.

INTRODUCTION

Vitamin D is well known for its role in calcium and bone metabolism; however, its deficiency may play a role in type 2 diabetes mellitus (T2DM).[1] The exact pathogenesis of T2DM remains unknown, but the condition is a result of different environmental and biochemical factors.[2] It is important then to look at different biochemical components to determine their role in T2DM. The biochemical component that is of particular interest in this study is vitamin D.

Cholecalciferol (vitamin D_3) is photosynthesized from 7-dehydroxycholecalciferol within the epidermal layer of the skin. When ultraviolet B (UVB) radiation from a source such as the sun strikes the skin, 7-dehydroxycholecalciferol transforms into vitamin D_3.[3 4] Vitamin D_3 undergoes hydroxylation in the liver to form calcifediol (25-hydroxyvitamin D). Calcifediol (25(OH)D) is further hydroxylated in the kidneys to form calcitriol (active form of vitamin D). Calcitriol (1,25-dihydroxyvitamin D_3) mediates its metabolic effect by binding to the Vitamin D Receptor (VDR) found inside the cell.[5] Calcitriol ($1,25(OH)_2D$) has a half-life of ~4 hours, so it is not effective in reflecting the overall vitamin D status of humans.[6] 25(OH)D has a minimal circulating half-life of 2 months since it can be stored and released from adipose and muscle tissue.[7 8] For the purposes of this study, 25(OH)D will be used to reflect the subjects' vitamin D levels.

All study participants are from the Caribbean, in the country of Trinidad (10.6667° N; 61.5167° W), which generally has a warm and sunny climate throughout the year. The study participants generally have skin type V (brown) according to the Fitzpatrick[9] classification of skin type. It is expected that most study participants

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experience sufficient sunlight which can result in participants having sufficient levels of 25(OH)D.[10 11] If a patient normally remains indoors, synthesis of vitamin D_3 from sunlight will be low but vitamin D can be obtained from fish, eggs, and vitamin D fortified milk.[10] Vitamin D deficiency and insufficiency are characterised as 25(OH)D <20 and 21–29 ng/mL, respectively.[6]

Studies have shown that T2DM and hypertension are related;[12–14] however, 25(OH)D's relation to blood pressure (BP) is unclear and the literature surveyed for 25(OH)D and BP gave conflicting reports of this relationship.[10 11 15] In this study, it was hypothesized that 25(OH)D levels were significantly lower in patients with T2DM and systolic BP (SBP) over 130 mm Hg.

RESEARCH DESIGN AND METHODS

Ethical approval to conduct the study was obtained from the University of the West Indies (UWI), St. Augustine, the South West Regional Health Authority (SWRHA), and the North Central Regional Health Authority (NCRHA) in Trinidad and Tobago. Subjects were randomly chosen at the Eric Williams Medical Sciences Complex (EWMSC) and San Fernando General Hospital (SFGH) in Trinidad. The sample size chosen for the study was 80 because of limitations in resources for assays. Both effect size and sample size with 80% power were estimated for future studies.

Hospital records were used to select at random patients who were diagnosed with T2DM. From the hospital records, patients with T2DM had a history of having glycated hemoglobin (HbA1c) and fasting blood glucose (FBG) values of ≥6.5% and ≥120 mg/dL, respectively. Clinicians at the SFGH and the EWMSC screened patients with T2DM again, to ensure that the patients did have the condition of T2DM before they were allowed to participate in the study. Controls were selected from the hospitals at random and were included in the study provided that the controls did not fall into the exclusion criteria. Antihypertensive drugs used and information on kidney disorders were recorded. The exclusion criteria for both controls and patients with T2DM were: persons consuming more than 8 mL of ethanol per week; taking multiple antihypertensive medications; having any form of cancer or any condition that may raise inflammatory markers; having T1DM; having any form of liver disease; having thyroid or parathyroid problems; being pregnant; and being under the age of 18. Additionally, exclusion criteria for controls only were HbA1c or FBG values of ≥6.5% or ≥120 mg/dL, respectively.

Subjects fasted and did not take any medication 8–10 hours before venous blood samples were drawn. On the morning of the blood draw, before venous samples were taken, the subjects' height and mass were measured. SBP and diastolic BP (DBP) were measured using a digital BP monitor. Venous blood samples drawn into blood collection tubes were centrifuged at 2000 g and separated into serum and plasma fractions. All blood fractions and two whole blood samples were stored at −70°C subsequent to analysis. Plasma 25(OH)D was determined by ELISA (ADI-900-215, Enzo Life Sciences, USA). Serum cholesterol was assayed using an automated dry chemistry analyzer (Cobas 6000, Roche Diagnostics, USA). Four subjects were removed from the study because of blood sample hemolysis. The final sample size for the study was 76 subjects (24 males and 52 females).

Software packages used for statistical analyses were IBM SPSS Statistics V.21, Minitab 16, and G*Power 3.1.7. Statistical analyses performed were Anderson-Darling test, independent t-test, Mann-Whitney U-test, Fisher's exact test, Spearman's correlation (r_s), logistic regression, and general linear model (GLM) univariate

Table 1 Demographic details of study sample

Parameter	All subjects, n=76 (%)	Controls, n=35 (%)	Patients with T2DM, n=41 (%)	25(OH)D>25 ng/mL, n=58 (%)	25(OH)D≤25 ng/mL, n=18 (%)
Age, years					
40–50	15 (20)	12 (34)	4 (10)	13 (22)	7 (39)
51–70	51 (67)	18 (52)	32 (78)	34 (59)	10 (55)
70–80	10 (13)	5 (14)	5 (12)	11 (19)	1 (6)
Ethnicity					
East Indian	43 (56)	16 (46)	27 (66)	31 (53)	12 (67)
African	22 (29)	14 (40)	8 (19)	20 (35)	2 (11)
Mixed	11 (15)	5 (14)	6 (15)	7 (12)	4 (22)
BMI, kg/m^2					
<25	15 (20)	10 (29)	5 (12)	11 (19)	4 (22)
25–30	26 (34)	11 (31)	15 (37)	19 (33)	7 (39)
>30	35 (46)	14 (40)	21 (51)	28 (48)	7 (39)
Subjects on antihypertensive medication	40 (53)	15 (43)	25 (61)	33 (57)	7 (39)
Kidney disease	7 (9)	5 (14)	2 (5)	7 (12)	0 (0)

BMI, body mass index; T2DM, type 2 diabetes mellitus.

Table 2 Characteristics of the variables in study in relation to T2DM

Variable	Mean±SD			Distribution	Test statistic	p Value	Effect sized	Sample size estimate, 80% power
	Total (n=76)	Controls (n=35)	Patients with T2DM (n=41)					
Age (years)	58.9±9.6	57.7±10.8	59.8±8.6	Normal	t	0.375	0.2	792
BMI (kg/m^2)	30.2±6.8	29.1±6.5	31.1±7.0	3P-Weibull	U	0.293	0.3	410
SBP (mm Hg)	145.6±22.7	141±20	149.8±24.2	Normal	t	0.095	0.4	200
DBP (mm Hg)	87.7±10.8	87±11	88.3±10.9	Normal	t	0.598	0.1	3162
25(OH)D (ng/mL)	38.3±17.8	41.3±18.6	35.7±16.9	3P-Weibull	U	0.139	0.3	410
Chol (mg/dL)	191.0±49.8	195.1±49.0	187.6±50.8	Normal	t	0.459	0.2	792

25(OH)D, vitamin D; BMI, body mass index; Chol, cholesterol; DBP, diastolic blood pressure; SBP, systolic blood pressure; t, independent t-test; T2DM, type 2 diabetes mellitus; U, Mann-Whitney U-test.

Table 3 Means of 25(OH)D, adjusted for gender and age

Subjects	Gender	Adjusted mean 25(OH)D, ng/mL	95% CI		p Value
			Lower bound	Upper bound	
Controls, n=35	Male, n=12	40.4	30.3	50.5	0.472
	Female, n=23	43.6	35.6	51.5	
Patients with T2DM, n=41	Male, n=12	36.3	24.8	47.8	
	Female, n=29	33.5	26.7	40.3	

T2DM, type 2 diabetes mellitus.

analysis. A p value <0.05 meant a statistically significant result. SBP was transformed via natural logarithm (ln) in order for the requirements of the GLM to be met.

RESULTS

Overall, no relationship was found among 25(OH)D, T2DM and use of specific anti-hypertensive agents. A significant relationship existed between 25(OH)D and SBP.

Table 1 gave the overall demographic details of subjects in the study. Table 2 displayed the characteristics of predictor variables in relation to the total sample size, controls, and patients with T2DM. The calculated effect sizes and sample size estimates for each variable were stated. These estimated sample sizes calculated in the last column of table 2 were not the sample size used in this study. The distributions given were for controls and for patients with T2DM independently. The test statistics used to compare differences between controls and T2DM for the various predictor variables were shown. Table 2 displayed no significant differences in age, body mass index, SBP, DBP, and 25(OH)D between controls and patients with T2DM. Table 3 displayed the means for 25(OH)D adjusted for age and gender whereby there was no significant difference in 25(OH)D between patients with T2DM and controls (p=0.472).

SBP was divided into two categorical variables for SBP >130 and ≤130 mm Hg, which were not displayed in a table. The average 25(OH)D for these categories were 39.3±19.3 and 26.5 ±11.8 ng/mL, respectively (p<0.001, 25(OH)D data were log-normal transformed to follow normal distribution). A Mann-Whitney U-test was also applied for the 25(OH)D between the SBP >130 and ≤130 mm Hg categories, which gave a significant difference (p<0.001). There was a moderately positive correlation between SBP and 25(OH)D (r_s=0.38, p=0.001).

Table 4 GLM output for ln (SBP) as a dependent variable

Source	Significance	Effect size (d)	Observed power (%)
Corrected model	0.006	0.4	86
T2DM/controls	0.046	0.2	52
Gender	0.558	0.1	9
25(OH)D>25 ng/mL	0.002*	0.4	89

*p<0.05=statistically significant.
GLM, general linear model; ln, natural logarithm; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Table 4 displayed the results of a GLM univariate analysis applied to ln (SBP) as a dependent variable with categorical variables T2DM/controls, gender, and 25(OH)D (>25 and ≤25 ng/mL) as fixed factors. There was a significant difference between 25(OH)D levels >25 and ≤25 ng/mL for the dependent variable ln (SBP).

Table 5 displayed the OR, adjusted for age, gender, and T2DM diagnosis, when separating 25(OH)D and BP into categorical variables. The 25(OH)D levels were divided into two categories for concentrations >30 and >25 ng/mL. SBP data were divided into two categories for SBP values >130 and >140 mm Hg. SBP/DBP data were divided into five categories for SBP/DBP >130/90, >130/100, >135/100, >140/80, and 140/90 mm Hg.

Table 5 Adjusted ORs for blood pressure and 25(OH)D

Blood pressure, mm Hg	Adjusted OR (95% CI) 25(OH)D>30 ng/mL	25(OH)D>25 ng/mL
SBP>130	4.7 (1.5 to 15.1) p=0.009*	7.9 (2.2 to 28.7) p=0.002*
SBP>140	2.0 (0.8 to 5.4) p=0.157	6.5 (1.8 to 24.2) p=0.005*
SBP/DBP>130/90	6.8 (1.8 to 25.4) p=0.005*	5.5 (1.5 to 19.8) p=0.009*
SBP/DBP>130/100	5.5 (1.7 to 18.3) p=0.005*	7.2 (2.0 to 26.0) p=0.003*
SBP/DBP>135/100	4.0 (1.4 to 11.5) p=0.010*	6.2 (1.8 to 22.0) p=0.004*
SBP/DBP>140/80	2.3 (0.9 to 6.4) p=0.096	5.8 (1.5 to 21.5) p=0.009*
SBP/DBP>140/90	2.3 (0.9 to 6.4) p=0.096	5.8 (1.5 to 21.5) p=0.009*

n=76; OR adjusted for Age, gender, T2DM diagnosis.
*p<0.05=statistically significant.
DBP, diastolic blood pressure; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Considering all the BP categories, all the ORs were significantly higher for 25(OH)D>25 ng/mL. Regarding 25(OH)D>30 ng/mL, all the ORs for BP categories were significantly higher with the exceptions of SBP/DBP >140/80 and >140/90 mm Hg. Subjects with SBP above 130 mm Hg had the highest odds of having 25(OH)D>25 ng/mL (OR 7.9 (2.2–28.7)).

In table 6, 25(OH)D was separated into two concentration categories, >25 and ≤25 ng/mL. Subjects were divided into categories taking and not taking antihypertensive medication as well as the type of antihypertensive medication used. There was no significant difference (p=0.181) between the 25(OH)D categories for patients using and not using antihypertensive medication. The table also displayed that there was no specific antihypertensive that was more associated with a change in 25(OH)D based on Fisher's exact test (p=0.386).

Table 6 Contingency table for 25(OH)D categories and antihypertensive therapy

Category	25(OH) D>25 ng/mL	25(OH) D≤25 ng/mL	p Value
Subjects not on antihypertensive drug therapy	25	11	0.181*
Subjects on antihypertensive drug therapy	33	7	
Antihypertensive drug used			
None	25	11	0.386†
ACE inhibitors	14	4	
β-Blockers	8	0	
Diuretics	1	1	
Calcium channel blockers	4	0	
α-Blockers	3	1	
Angiotensin receptor blockers	1	1	
Other drugs	2	0	

*χ^2 Test.
†Fisher's exact test.

DISCUSSION

In table 2, the results of the Mann-Whitney U-test for T2DM and controls indicated no significant difference in 25(OH)D between patients with T2DM and controls (p=0.139). After adjustments for gender and age were made, table 2 also indicated no significant differences in 25(O5)D levels between patients with T2DM and controls (p=0.472). The tropical island of Trinidad, in which the subjects reside, generally has a sunny climate which may account for the production of vitamin D being similar in both groups. Vitamin D is synthesized when sunlight strikes the skin, resulting in the conversion of 7-dehydroxycholesterol to vitamin D_3.[4] Studies have demonstrated that low 25(OH)D levels are related to T2DM susceptibility; thus, for the Trinidadian population, the problem with the vitamin D axis is that there may be a problem with the VDR. The VDR gene polymorphism may cause subtle changes in the three-dimensional conformation of VDRs. These subtle conformational changes may result in individuals having VDRs with different affinities toward $1,25(OH)_2D$. The differences in affinities may account for an individual's susceptibility toward T2DM. It can be hypothesized then that someone with VDR receptors of low $1,25(OH)_2D$ affinity is susceptible to T2DM. Thus, further investigations are required to elucidate the VDR polymorphisms, which may cause variance in VDRs, in relation to T2DM. This study does not provide conclusive evidence of a relationship existing between VDR and T2DM.

The moderately positive correlation between 25(OH)D and SBP (r_s=0.38, p=0.001) was not expected since a majority of the literature demonstrated either no relationship or an inverse correlation between 25(OH)D and SBP.[10] The positive correlation between 25(OH)D and SBP does agree with a few studies, most of which have small sample sizes.[16–19] Coupled to this unexpected correlation, patients with SBP>130 mm Hg were eight times more likely to have a 25(OH)D>25 ng/mL. Further investigations in tropical regions are required to determine if these significant findings solely apply to inhabitants in these regions.

The study undertaken did not meet the requirements of the estimated sample size, so it would be noteworthy

to expand the sample size in order to effectively draw a better conclusion on the correlation between 25(OH)D and SBP in the Trinidadian population. The GLM univariate analysis displayed in table 4 compensated for the weakness in sample size when considering the 25(OH)D categories >25 and ≤25 ng/mL in relation to ln SBP (p=0.002, 89% power). The 25 ng/mL 25(OH)D categories were examined in relation to the use of a specific antihypertensive; however, table 6 displayed that there was no significant relationship. This may indicate that antihypertensive medication did not influence a change in vitamin D levels.

The study is a pilot study, which would enable researchers to better determine future sample sizes with sufficient power in relation to a particular outcome variable of interest. There is some obvious complexity in relating T2DM to the vitamin D axis. Based on current studies and the pilot study conducted, it seems that 25(OH)D cannot be used as a biochemical marker or predictor for T2DM, but there is some role of vitamin D in the pathogenesis of T2DM. Further elucidation of the binding interaction of vitamin D to VDR as well as VDR polymorphisms is required to obtain clarity on a possible relationship between T2DM and the vitamin D axis.

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