The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2022; 29: 242-251. http://doi.org/10.5551/jat.59113

Low Vitamin D Levels are Associated with Vascular Endothelial Dysfunction in Patients with Poorly Controlled Type 2 Diabetes: A Retrospective Study

Kenichi Tanaka, Yosuke Okada, Maiko Hajime and Yoshiya Tanaka

The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, Kitakyusyu, Japan

Aim: This study aimed to determine the association between serum 25-hydroxyvitamin D (25(OH)D) levels and vascular endothelial function in patients with type 2 diabetes (T2D).

Methods: This retrospective study included 113 patients with poorly controlled T2D who were admitted for in-hospital diabetes educational program and underwent measurements of serum 25(OH)D levels and reactive hyperemia index (RHI).

Results: Serum 25(OH)D levels significantly correlated with RHI in T2D patients. Receiver operating characteristic (ROC) curve analysis showed that serum 25(OH)D level of 16.5 ng/mL is the optimal cutoff level for predicting vascular endothelial dysfunction (RHI < 1.67), with a sensitivity of 68.5%, specificity of 67.9%, and area under the ROC curve of 0.668 (95% confidence interval [CI]: 0.566–0.770, $p=0.002$). The mean RHI was significantly lower (1.70 ± 0.54) in patients with low 25(OH)D levels ($n=56$, 25(OH)D levels < 16.5 ng/mL) than that (1.99 ± 0.58; $p<0.001$) in patients with high 25(OH)D levels ($n=57$, 25(OH)D level ≥ 16.5 ng/mL). The proportion of patients with RHI < 1.67 was higher in the low 25(OH)D group than in the high 25(OH)D group (38% vs. 18%; $p<0.001$). Multivariate logistic regression analysis identified that serum 25(OH)D level < 16.5 ng/mL was associated with increased odds of RHI < 1.67 (odds ratio 4.598, 95% CI 1.961–10.783, $p<0.001$).

Conclusion: The results demonstrated the association of serum 25(OH)D levels with endothelial function in poorly controlled T2D patients and identified serum 25(OH)D level of < 16.5 ng/mL as a predictor of RHI < 1.67. Serum 25(OH)D level is a potentially useful marker of vascular endothelial dysfunction in poorly controlled T2D patients.

Key words: 25-hydroxyvitamin D, Reactive hyperemia index, Type 2 diabetes, Vascular endothelial function

Introduction

Patients with type 2 diabetes (T2D) are at high risk of macroangiopathy than non-diabetics, with approximately two to six times higher risks of myocardial infarction and death related to ischemic heart disease and two to three times higher risk of cerebral infarction[1-3]. Such high cardiovascular risk cannot be fully explained by traditional risk factors only, such as age, sex, hypertension, dyslipidemia, and smoking, but it seems to be also associated with endothelial dysfunction, microalbuminuria, and inflammation[4]. Evidence shows that vascular endothelial dysfunction occurs at an early stage of arteriosclerosis[5] and that in T2D, vascular endothelial dysfunction can be observed during early glucose intolerance[6]. It is therefore important to assess endothelial function for the early detection of arteriosclerosis.

Vitamin D plays an important role in calcium homeostasis and bone metabolism through the regulation of calcium absorption from the intestine,

Address for correspondence: Yoshiya Tanaka, The First Department of Internal Medicine, School of Medicine University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka, Yahatanishi-ku Kitakyushu, Fukuoka 807-8555, Japan E-mail: tanaka@med.uoeh-u.ac.jp

Received: July 4, 2020 Accepted for publication: November 17, 2020

and its deficiency has been linked to all-cause mortality, cardiovascular mortality, and heart failure[7, 8]. T2D patients have lower serum 25-hydroxyvitamin D (25(OH)D) levels than non-diabetics[9-12]. T2D patients with vitamin D deficiency also have limited brachial flow-mediated dilation (FMD)[13]. FMD measurement has been traditionally used for the assessment of vascular endothelial function. Recently, a new device (EndoPAT 2000, Itamar Medical, Caesarea, Israel) using peripheral arterial tonometry (PAT), with established objectivity and reproducibility, has been increasingly employed a noninvasive method for the assessment of vascular endothelial function[14]. Moreover, the usefulness of reactive hyperemia index (RHI) in predicting vascular diseases has been previously reported[15]. However, clinical studies using RHI in T2D patients are limited than those using FMD, and only a few studies have investigated the association between vitamin D status and RHI in T2D patients.

Aim

This study aimed to determine the association between serum vitamin D levels and vascular endothelial function as assessed by RHI in T2D patients.

Methods

Patients

Of the patients with T2D aged over 20 years who were admitted to the Hospital of the University of Occupational and Environmental Health, Japan, for in-hospital diabetes educational program between April 2014 and December 2018, this study analyzed those who underwent measurements for serum 25(OH)D levels and reactive hyperemia index (RHI). The following patients were excluded: those with type 1 diabetes mellitus, severe infection or serious trauma, and renal dysfunction (estimated glomerular filtration rate [eGFR] of <30 mL/min/1.73 m^2), those being treated at that time for osteoporosis, those using calcium and/or vitamin D supplements, and those found to have abnormal hormone profile (excluding those with hormone levels maintained within the normal range by treatment).

Diabetic complications were evaluated in this study as follows: diabetic retinopathy was diagnosed based on the results of funduscopic examination performed by expert ophthalmologists and classified according to the Davis classification into no diabetic, simple, pre-proliferative, and proliferative retinopathy. Diabetic nephropathy was considered positive in patients with urinary albumin excretion rate (presented as urinary albumin-to-creatinine ratio [UACR]) of ≥ 30 mg/g creatinine and/or an eGFR of <30 mL/1.73 m^2, in accordance with the Classification of Diabetic Nephropathy 2014 in Japan[16]. Diabetic neuropathy was diagnosed by the presence of two or more clinical symptoms (bilateral spontaneous pain, hypoesthesia, or paresthesia of the legs), absence of Achilles tendon reflexes, and decreased vibration sensations in response to a C128 tuning fork. Patients who had already been diagnosed with coronary heart disease, cerebrovascular disease, or arteriosclerosis obliterans were considered to have macrovascular complications at the time of enrollment.

The study protocol was approved by the ethics committee of the University of Occupational and Environmental Health, Japan (Approval No. H27-186), and informed consent was obtained from all participants.

Study Design

On admission, we collected patient data including age, sex, blood pressure, body mass index (BMI), duration of diabetes, presence of diabetic microangiopathy or macroangiopathy, presence of hypertension, presence of dyslipidemia, antidiabetic drug use, antihypertensive drug use, antilipidemic drug use, and the smoking status (never, former, current). The levels of fasting plasma glucose, HbA1c, fasting plasma insulin (FPI), serum C peptide (CPR), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured on the second or third hospital day. Areas of subcutaneous and visceral adipose tissue were measured on computed tomography (CT). In addition, vascular endothelial function was evaluated under fasting conditions within 4 days of hospitalization, using a PAT device (EndoPAT2000, Itamar Medical).

Biochemical and Clinical Measurements

HbA1c levels (%) were measured using a high-performance liquid chromatography method with a Tosoh HLC-723 G8 analyzer (Tosoh Co., Kyoto, Japan) and expressed in National Glycohemoglobin Standardization Program (NGSP) equivalent values calculated from the following equation: HbA1c (NGSP) = HbA1c (Japan Diabetes Society [JDS]) (%) + 0.4%[17]. The homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated using the following formula: FPG (m/dL) × FPI (μU/mL)/405. The homeostasis model assessment of β-cell function (HOMA-β) values were calculated using the following formula: FPI (μU/mL) × 360/(FPG [mg/dL]

−63). The CPR index was calculated using the following formula: CPR (ng/mL)/FPG (m/dL) × 100. Total homocysteine levels were measured using high-performance liquid chromatography (SRL Co., Tokyo, Japan). Carotid intima-media thickness (IMT) was measured by a well-trained medical technologist from the Hospital of the University of Occupational and Environmental Health, Japan. The highest IMT value was defined as the maximum IMT. The mean values of the right and left maximum IMT were used for statistical analysis. If a plaque was present, it was included in the IMT measurement. Serum 25(OH)D levels were measured using the DIASource 25OH Vitamin D total-RIA-CT Kit (DIAsource ImmunoAssay Co., Louvain-la-Neuve, Belgium) by radioimmunoassay. A 25(OH)D level <20 ng/mL indicated vitamin D deficiency[18].

Noninvasive Vascular Function Test

We used the PAT-based method for digital assessment of vascular endothelial function as previously described in detail[19]. Briefly, after an acclimatization period of 30 min (before breakfast) in a temperature- and light-controlled room, the baseline pulse amplitude was recorded during a period of 5 min before the induction of ischemia. Ischemia was induced by placing the sphygmomanometer cuff on the upper arm, while the opposite arm served as the control. The PAT probes were placed on one finger of each hand. After 5 min, the blood pressure cuff was inflated to 60 mmHg above the systolic pressure or to 200 mmHg for 5 min and then deflated to induce reactive hyperemia. As a measure of reactive hyperemia, the RHI was calculated as the ratio of the average amplitude of the PAT signal over 1 min beginning 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the average amplitude of the PAT signal over the 2.5-min period before cuff inflation (baseline) (control arm, B; occluded arm, D). Thus, the following formula was used: RHI = (C/D)/(A/B) × baseline correction. In this study, an RHI < 1.67 was used to define vascular endothelial dysfunction[20, 21].

Statistical Analysis

Data are expressed as mean ± standard deviation. Data distribution was determined using the Shapiro–Wilk test. Categorical values were tested by the $-\chi^2$ test. Comparison of the characteristics of T2D patients with low and high vitamin D levels was carried out using the unpaired *t*-test for normally distributed data and the Mann–Whitney test for data with skewed distribution. Correlation analyses between serum 25(OH)D levels and the baseline characteristics of patients were performed using Pearson's correlation analysis for normally distributed variables and Spearman's correlation analysis for variables with skewed distribution. To investigate the predictive value of serum 25(OH)D levels for vascular endothelial dysfunction in patients with T2D, receiver operating characteristic (ROC) curves were plotted to determine the optimal cutoff value of serum 25(OH)D level. Moreover, we performed univariate and multivariate logistic regression analyses. In the multivariate logistic regression analysis, the independent variables were chosen from age, sex, and other factors with *p* values of less than 5% in the univariate logistic regression analysis, after excluding factors showing multicollinearity on Spearman's correlation analysis. Data were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were carried out using SPSS version 25.0 (SPSS Inc., Chicago, IL). A *p* value less than 0.05 was considered to denote statistical significance.

Results

Patient Demographics

A total of 113 patients with poorly controlled T2D were enrolled in the study. The baseline characteristics of the study patients are summarized in **Table 1**. The mean disease duration was 7.3 ± 8.0 years and the mean HbA1c level was 9.3 ± 1.9%. The mean serum 25(OH)D level was 18.4 ± 8.1 ng/mL. The proportion of patients with vitamin D deficiency and vascular endothelial dysfunction (RHI <1.67) was 66.4% and 49.6%, respectively.

Relationship between Serum 25(OH)D Levels and Baseline Characteristics

Table 2 summarizes the correlation coefficients between 25(OH)D levels and the baseline characteristics of T2D patients. There was a significant correlation between serum 25(OH)D level and RHI (r = 0.285, $p = 0.002$, **Fig. 1**). Serum 25(OH)D levels also correlated significantly with age and HDL-C levels and negatively with BMI, HbA1c, FPI, and CPR, UACR, subcutaneous adipose tissue areas, and visceral adipose tissue areas.

Characteristics of Patients with Low Vitamin D Levels

The ROC curve analysis identified 16.5 ng/mL as the optimal cutoff level of serum 25(OH)D for the prediction of RHI <1.67 in T2D patients, with sensitivity of 68.5%, specificity of 67.9%, and an area under the ROC curve of 0.668 (95% CI: 0.566–0.770). Based on this cutoff value, the patients were

Table 1. Baseline characteristics of T2D patients

n	113
Age (years)	59.8 ± 12.6
Sex (men/women)	64/49
Duration of diabetes (year)	7.3 ± 8.0
Body mass index (kg/m^2)	27.5 ± 6.1
Systolic blood pressure (mmHg)	132.4 ± 15.6
Diastolic blood pressure (mmHg)	78.9 ± 11.6
FPG (mg/dL)	157.4 ± 43.0
HbA1c (%)	9.3 ± 1.9
FPI (μg/mL)	9.2 ± 6.1
HOMA-IR	3.5 ± 2.2
HOMA-β (%)	43.2 ± 39.9
S-CPR (ng/mL)	2.5 ± 1.3
CPR index	1.6 ± 0.9
eGFR (mL/min/1.73 m^2)	79.2 ± 22.6
UACR (mg/g Cre)	131.5 ± 396.6
LDL-C (mg/dL)	109.1 ± 38.3
HDL-C (mg/dL)	47.3 ± 11.9
TG (mg/dL)	155.0 ± 87.6
Calcium (mg/dL)	9.5 ± 0.4
Phosphate (mg/dL)	3.6 ± 0.5
25(OH)D (ng/mL)	18.4 ± 8.1
Vitamin D deficiency (%)	75 (66.4)
Subcutaneous adipose tissue areas (cm^2)	218.4 ± 127.6
Visceral adipose tissue areas (cm^2)	186.4 ± 75.9
Carotid IMT (mm)	1.0 ± 0.4
Carotid plaque (%)	71 (63.4)
Total homocysteine (nmol/L)	10.7 ± 4.3
RHI	1.85 ± 0.58
RHI < 1.67 (%)	56 (49.6)
Hypertension (%)	81 (71.7)
Dyslipidemia (%)	85 (75.2)
Antihypertensive drug (%)	51 (45.1)
Antilipidemic drug (%)	40 (35.4)
Smoking status (never/former/current; %)	45/28/27
Diabetes therapy	
No medication (%)	46 (40.7)
DPP-4 inhibitor (%)	47 (39.8)
Sulfonylurea (%)	30 (26.5)
Glinide (%)	2 (1.8)
Biguanide (%)	28 (24.8)
Thiazolidine (%)	6 (5.3)
α-glucosidase inhibitor (%)	5 (4.4)
SGLT-2 inhibitor (%)	3 (2.7)
Insulin (%)	17 (15.0)
GLP-1 receptor agonist (%)	6 (0.5)
Diabetic microvascular complications	
Retinopathy (%)	41 (36.3)
Nephropathy (%)	35 (31.0)
Neuropathy (%)	43 (38.0)
Diabetic macrovascular complications	
Coronary heart disease (%)	11 (9.7)
Cerebrovascular disease (%)	6 (5.3)
Arteriosclerosis obliterans (%)	0 (0.0)

Data are mean ± standard deviation, or *n* (%).
T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cell function; S-CPR, serum C peptide, CPR index, Serum C-peptide index; EGFR, estimated glomerular filtration rate; UACR; urinary albumin-to-creatinine ratio, LDL-C, low-density cholesterol; HDL-C, high-density cholesterol; TG, triglyceride; 25(OH) D, 25-hydroxyvitamin D; Carotid IMT, Carotid intima-media thickness; RHI, reactive hyperemia index; DPP-4, dipeptidyl peptidase-4; SGLT-2, Sodium-glucose cotransporter 2; GLP-1, glucagon-like peptide-1.

Table 2. Correlation coefficients between 25(OH)D levels and the baseline characteristics of T2D patients

	T2D patients	
	r	*P*-value
Age	0.245	0.009
Duration of diabetes	0.049	0.610
Body mass index	-0.248	0.008
Systolic blood pressure	0.046	0.625
Diastolic blood pressure	0.077	0.419
FPG	0.014	0.883
HbA1c	-0.193	0.041
FPI	-0.315	0.002
HOMA-IR	-0.313	0.002
HOMA-β	-0.243	0.017
S-CPR	-0.195	0.040
CPR index	-0.133	0.162
eGFR	-0.083	0.384
UACR	-0.219	0.020
LDL-C	-0.056	0.560
HDL-C	0.200	0.033
TG	-0.120	0.206
Calcium	0.072	0.450
Phosphate	-0.141	0.135
Subcutaneous adipose tissue areas	-0.318	0.001
Visceral adipose tissue areas	-0.194	0.040
Carotid IMT	0.028	0.769
Total homocysteine	-0.128	0.184
RHI	0.285	0.002

Data are results of Pearson correlation analysis for normally distributed variables and Spearman rank correlation for variables with skewed distribution. Abbreviations as in Table 1.

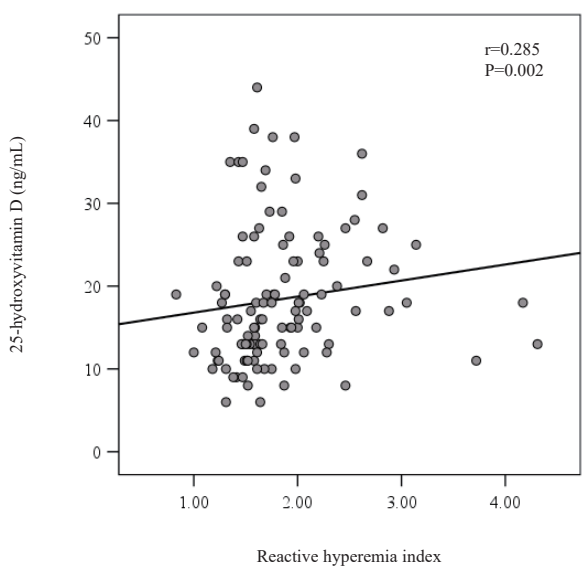

Fig. 1. Relationship between 25(OH)D levels and RHI in T2D patients

25(OH)D, 25-hydroxyvitamin D; RHI, reactive hyperemia index; T2D, type 2 diabetes

divided into those with low vitamin D levels (25(OH)D <16.5 ng/mL) and those with high vitamin D levels (25(OH)D ≥ 16.5 ng/mL), and their characteristics were compared (Table 3). The mean serum 25(OH)D level of the low vitamin D group was almost 50% (12.2±2.6 ng/mL) of that of the high vitamin D group (24.6±6.9 ng/mL). Patients of the low vitamin D group were significantly younger, had lower HDL-C levels, and had significantly higher values of BMI, FPI, CPR, subcutaneous adipose tissue areas, visceral adipose tissue areas, and total homocysteine than those of the high vitamin D group. The low vitamin D group also showed significantly lower RHI values than the high vitamin D group (1.70±0.54 vs. 1.98±0.58; $p<0.001$, Fig. 2). The proportion of patients with RHI <1.67 was also higher in the low vitamin D group than in the high vitamin D group (38% vs. 18%; $p<0.001$).

Association between Serum 25(OH)D Levels and Vascular Endothelial Dysfunction

Finally, we investigated the association between vitamin D levels and RHI <1.67. Univariate logistic regression analysis showed a close association between serum 25(OH)D levels and RHI <1.67, with an OR of 0.945 (95% CI: 0.899–0.993, $p=0.025$). However, multivariate logistic regression analysis including age, sex, and other significant factors selected by univariate analysis based on $p<0.05$ identified that carotid IMT and no use of diabetic medications, but not serum 25(OH)D levels, were significantly associated with RHI <1.67. When "serum 25(OH)D levels <16.5 ng/mL" was used instead of general serum 25(OH)D levels, it was associated with increased odds of RHI <1.67, even after adjustment for various confounding factors (OR 4.598, 95% CI 1.961–10.783, $p<0.001$, Table 4).

Discussion

The main finding of this study is the significant correlation between serum 25(OH)D levels and RHI in poorly controlled T2D patients; in particular, low level of serum 25(OH)D (less than 16.5 ng/mL) was an independent risk factor for endothelial dysfunction and was associated with more than fourfold increase in the risk of vascular endothelial dysfunction. Considering the high incidence of cardiovascular events in patients with T2D and the possible association between low vitamin D levels and increased risk of cardiovascular events due to the progression of vascular endothelial dysfunction, we advocate active measurement of vitamin D levels in patients with T2D and assessment of vascular endothelial function in vitamin D-deficient patients. Compared to FMD measurement, which is conventionally used for the assessment of vascular endothelial function in clinical practice, RHI, which was used in this study, is advantageous in that it is a simple test that does not require high level of skills. Moreover, one of the major conclusions of the Framingham Heart Study was that the EndoPAT-based RHI measurement is a useful measure of peripheral vascular function[22]. To this effect, both RHI and FMD are useful measures of vascular endothelial function and can predict cardiovascular events; however, they do not correlate with each other and have been shown to be associated with different risk factors[23]. With the exception of one study that examined the beneficial effects of vitamin D supplementation on RHI[24], there is little or no information on the association between vitamin D levels and RHI in T2D patients. To our knowledge, although our study was retrospective in nature, it was the first to demonstrate the association of low 25(OH)D levels and RHI in T2D patients.

What are the cellular mechanisms behind the effects of vitamin D on vascular endothelial function? While our study did not directly examine these mechanisms, we postulate the following scenarios: First, calcitriol, an active form of vitamin D, enhances angiogenic responses, such as endothelial repair by promoting the differentiation of monocytes into myeloid angiogenic cells; second, calcitriol augments endothelial function by increasing endothelial nitric oxide (NO) synthase expression[25]; and third, vitamin D can also improve vascular endothelial function through the regulation of vascular smooth muscle cell proliferation and chronic inflammation[26].

In this study, vitamin D deficiency, defined by a 25(OH)D level <20 ng/mL, was observed in 66% of T2D patients. It is well documented that T2D patients and obese individuals tend to have lower serum 25(OH)D levels[9-12]. A possible explanation for this is that obese individuals may have poor exercise habits and less exposure to ultraviolet light. Moreover, after exposure to sunlight, the increase in the serum concentration of 25-vitamin D was 57% lower among obese subjects than non-obese subjects, independent of the amount of cutaneous precursor of vitamin D3[12]. This study also showed lower HDL-C levels and higher BMI, insulin resistance, and subcutaneous/visceral adipose tissue areas in the low vitamin D group (Table 3), suggesting an association between hypovitaminosis D and metabolic abnormalities, which may contribute to vascular endothelial dysfunction.

A previous 5-year observational study of T2D

Table 3. Results of comparison between T2D patients with low and high vitamin D levels

	High 25(OH)D	Low 25(OH)D	*P*
N	57	56	
Age (years)	62.3 ± 11.5	57.4 ± 13.2	0.038
Sex (men/women)	36/21	28/28	0.158
Duration of diabetes (year)	7.8 ± 8.6	7.2 ± 7.3	0.936
Body mass index (kg/m^2)	26.2 ± 5.6	28.7 ± 6.4	0.018
Systolic blood pressure (mmHg)	132.9 ± 14.8	132.0 ± 16.6	0.425
Diastolic blood pressure (mmHg)	79.6 ± 10.2	78.0 ± 12.9	0.337
FPG (mg/dL)	156.6 ± 44.2	157.4 ± 43.0	0.947
HbA1c (%)	9.0 ± 1.9	9.5 ± 1.8	0.114
FPI (μg/mL)	7.3 ± 4.5	11.1 ± 6.9	0.002
HOMA-IR	2.8 ± 1.7	4.2 ± 2.4	0.001
HOMA-β (%)	35.9 ± 34.8	50.5 ± 43.6	0.028
S-CPR (ng/mL)	2.2 ± 1.2	2.7 ± 1.3	0.016
CPR index	1.5 ± 0.9	1.8 ± 0.9	0.097
eGFR (mL/min/1.73 m^2)	78.8 ± 23.6	73.8 ± 27.7	0.518
UACR (mg/g Cre)	50.7 ± 152.1	213.7 ± 532.1	0.080
LDL-C (mg/dL)	121.2 ± 39.9	119.3 ± 39.1	0.798
HDL-C (mg/dL)	50.5 ± 12.3	44.0 ± 9.8	0.008
TG (mg/dL)	145.8 ± 84.2	164.4 ± 90.8	0.129
Calcium (mg/dL)	9.7 ± 0.4	9.7 ± 0.3	0.159
Phosphate (mg/dL)	3.5 ± 0.5	3.6 ± 0.5	0.090
25(OH)D (ng/mL)	24.6 ± 6.9	12.2 ± 2.6	< 0.001
Subcutaneous adipose tissue areas (cm^2)	193.7 ± 120.5	243.5 ± 130.7	0.017
Visceral adipose tissue areas (cm^2)	171.2.9 ± 72.8	201.8 ± 76.6	0.016
Carotid IMT (mm)	1.0 ± 0.5	1.0 ± 0.4	0.850
Carotid plaque (%)	31 (55.4)	40 (71.4)	0.078
Total homocysteine (nmol/L)	9.8 ± 3.3	11.5 ± 5.1	0.040
RHI	1.99 ± 0.58	1.70 ± 0.54	< 0.001
RHI < 1.67 (%)	18 (31.6)	38 (67.9)	< 0.001
Hypertension (%)	38 (66.7)	43 (76.8)	0.233
Dyslipidemia (%)	43 (75.4)	42 (75.0)	0.957
Antihypertensive drug (%)	19 (33.3)	32 (57.1)	0.011
Antilipidemic drug (%)	20 (35.7)	20 (35.1)	0.944
Smoking status (never/former/current; %)	36/30/34	55/25/20	0.091
Diabetes therapy			
No medication (%)	27 (47.4)	19 (33.9)	0.146
DPP-4 inhibitor (%)	18 (31.6)	25 (44.6)	0.153
Sulfonylurea (%)	14 (24.6)	16 (28.6)	0.629
Glinide (%)	2 (3.5)	0 (0.0)	0.252
Biguanide (%)	14 (24.6)	14 (25.0)	0.957
Thiazolidine (%)	4 (7.0)	2 (3.6)	0.348
α-glucosidase inhibitor (%)	1 (1.8)	4 (7.1)	0.176
SGLT-2 inhibitor (%)	0 (0.0)	3 (4.9)	0.118
Insulin (%)	8 (14.0)	9 (16.1)	0.762
GLP-1 receptor agonist (%)	3 (5.3)	3 (5.4)	0.982
Diabetic microvascular complications			
Retinopathy (%)	18 (31.6)	23 (41.1)	0.294
Nephropathy (%)	15 (26.3)	20 (35.7)	0.280
Neuropathy (%)	23 (40.4)	20 (35.7)	0.612
Diabetic macrovascular complications			
Coronary heart disease (%)	4 (7.0)	7 (12.5)	0.326
Cerebrovascular disease (%)	2 (3.5)	4 (7.1)	0.331
Arteriosclerosis obliterans (%)	0 (0.0)	0 (0.0)	-

Data are mean ± standard deviation, or *n* (%).

P values by the paired *t*-test for normally distributed data and Wilcoxon signed-rank test for data with skewed distribution. Categorical values were tested by $-\chi^2$ test. *P* values are for differences between the two groups. Abbreviations as in Table 1

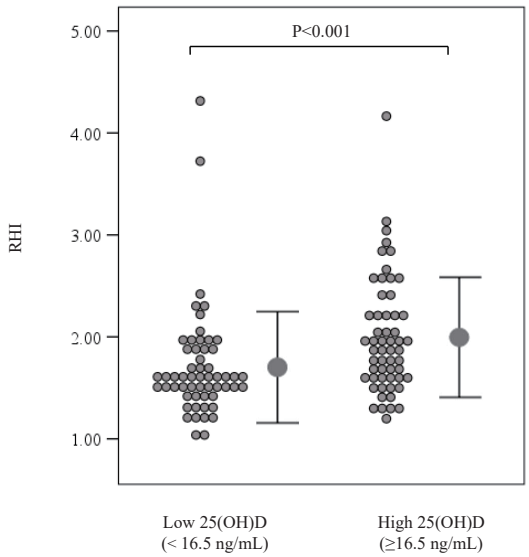

Fig. 2. Comparison of RHI between T2D patients with low and high vitamin D levels

Symbols represent individual data and circles with lines are group mean ± SD values.
25(OH)D, 25-hydroxyvitamin D; RHI, reactive hyperemia index; T2D, type 2 diabetes

Table 4. Binary logistic regression analyses of variables contributing to RHI < 1.67 in type 2 diabetes patients

	Univariate logistic regression analysis			Multiple logistic regression analysis		
	Wald χ^2	*P*	OR (95% CI)	Wald χ^2	*P*	OR (95% CI)
Intercept						
Age	0.058	0.809	0.996 (0.967-1.026)			
Women	0.012	0.914	0.960 (0.456–2.021)			
25(OH)D	4.997	0.025	0.945 (0.899–0.993)			
Dyslipidemia	4.359	0.037	2.611 (1.061–6.429)			
Carotid IMT	4.564	0.033	2.747 (1.087-6.941)	4.074	0.044	2.712 (1.029-7.147)
No use of diabetic medication	8.628	0.003	0.307 (0.139-0.675)	8.587	0.003	0.133 (0.133-0.670)
25(OH)D < 16.5[†]	14.176	< 0.001	4.574 (2.073-10.093)	12.307	< 0.001	4.598 (1.961-10.783)

Age, sex, and factors with $P < 0.05$ on univariate logistic regression were included in this multiple logistic regression.
[†]Serum 25(OH)D levels < 16.5 (ng/mL) as a variable instead of 25(OH)D in another multiple logistic regression analysis model

patients identified hypovitaminosis D to be associated with increased risks of microvasculopathies and macrovasculopathies[27]. Moreover, high hazard ratios of severe vitamin D deficiency were identified for all-cause (2.03 [95% CI 1.31–3.11]) and cardiovascular mortality (1.90 [95% CI 1.16–3.10]) in T2D patients[28]. These findings highlight the clinical importance of hypovitaminosis D in T2D patients. It is noteworthy that our study showed that serum vitamin D level had no significant effect on the incidence of microvasculopathies or macrovasculopathies. The discrepancy in this finding between our study and the above previous studies is probably due to the differences in the sample size, racial composition, and study design. In contrast, a significant correlation was observed between RHI and serum 25(OH)D levels, suggesting that vascular endothelial dysfunction due to vitamin D deficiency can occur in relatively early stages of T2D. The ROC curve analysis used in this study found that the cutoff level of 25(OH)D for predicting an RHI < 1.67 was 16.5 ng/mL (OR 4.598, 95% CI 1.961–10.783, $p < 0.001$; **Table 4**). Moreover, serum 25(OH)D level of < 20 ng/mL was identified by the multivariate logistic regression analysis to be an independent predictor of RHI < 1.67 (OR 2.574, 95% CI 1.043–6.355, $P = 0.040$), suggesting that vitamin D deficiency defined as 25(OH)D levels < 20 ng/mL is associated with vascular endothelial dysfunction.

It is important to determine whether vitamin D

supplementation can ameliorate vascular endothelial dysfunction in vitamin D-deficient T2D patients. Among the limited number of studies using RHI, one study showed that treatment of vitamin D deficiency with 2,000 or 4,000 IU of cholecalciferol for 16 weeks resulted in only a borderline increase in RHI ($p=0.07$) in T2D patients[24]. Inconsistent results have also been obtained using the FMD, with some studies demonstrating improvement[29] and others reported no changes[30, 31]. In a recent meta-analysis study investigating the effects of supplemental vitamin D on endothelial function, a sub-analysis involving T2D patients with vitamin D supplementation showed no significant improvement in FMD[32]. Meanwhile, in the D2d study, which evaluated the effects of vitamin D supplementation in individuals with prediabetes, although the risk of T2D was not reduced in the entire population, a 62% reduction in diabetes risk was observed in a subgroup of patients with a 25(OH) D level <12 ng/mL[33]. Considering this result, we suggest that vitamin D supplementation seems to have a positive effect on vascular endothelial function in vitamin D-deficient T2D patients, thus warranting further research on this important issue.

This study has several limitations. First, it did not include a control group of non-diabetics. However, it has been documented that serum 25(OH) D levels are lower in diabetics than in non-diabetics[9-12]. Second, patients with T2D included in this study were admitted for in-hospital educational program due to poorly controlled diabetes, and it is unclear whether the findings obtained in this study can be generalized to all patients with T2D. Third, sample size was small due to the retrospective design and the lack of consideration of seasonal variations in 25(OH)D measurements. Moreover, an increase in post-prandial glucose (PPG) level is one of the risk factors for endothelial dysfunction in patients with T2D; however, it could not be analyzed in the present study. Finally, this study did not consider whether antidiabetic treatment has a positive effect on vascular endothelial function and vitamin D levels in patients with diabetes. This issue should be investigated in future studies.

Conclusion

In conclusion, we have demonstrated in this study a significant correlation between 25(OH)D levels and RHI in poorly controlled T2D patients; in particular, low serum 25(OH)D levels (<16.5 ng/mL) were an independent risk factor for endothelial dysfunction and were associated with more than fourfold increase in the risk of vascular endothelial dysfunction. Our data suggest that serum 25(OH)D is a potentially useful biomarker for vascular endothelial dysfunction in poorly controlled T2D patients.

Acknowledgements

Not applicable.

Notice of Grant Support

None of the authors received specific financial support for this article.

Conflict of Interest

The authors declare no conflict of interest.

References

1) Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med, 1998; 339: 229-234
2) Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes care, 1993; 16: 434-444
3) Kannel WB, McGee DL: Diabetes and cardiovascular disease: The Framingham study. JAMA, 1979; 241: 2035-2038
4) Fonseca V, Desouza C, Asnani S, Jialalet I: Nontraditional risk factors for cardiovascular disease in diabetes. Endocr Rev, 2004; 25: 153-175
5) Ross R: Atherosclerosis--an inflammatory disease. N Engl J Med, 1999; 340: 115-126
6) Xu J, Zou M-H: Molecular insights and therapeutic targets for diabetic endothelial dysfunction. Circulation, 2009; 120: 1266-1286
7) Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W: Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Arch Intern Med, 2008; 168: 1340-1349
8) Pilz S, März W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H: Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab, 2008; 93: 3927-3935
9) Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G: Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. Diabetes Care, 2006; 29: 722-724
10) Hyppönen E, Power C: Vitamin D status and glucose

homeostasis in the 1958 British birth cohort: the role of obesity. Diabetes Care, 2006; 29: 2244-2246
11) Holick MF: Vitamin D deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. Nat Rev Endocrinol, 2011; 7: 73-75
12) Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF: Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr, 2000; 72: 690-693
13) Yiu YF, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Cheung BM, Tse HF: Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. J Clin Endocrinol Metab, 2011; 96: E830-835
14) Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, Pumper GM, Lerman LO, Lerman A: Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J, 2010; 31: 1142-1148
15) Matsue Y, Yoshida K, Nagahori W, Ohno M, Suzuki M, Matsumura A, Hashimoto Y, Yoshida M: Peripheral microvascular dysfunction predicts residual risk in coronary artery disease patients on statin therapy. Atherosclerosis, 2014; 232: 186-190
16) Haneda M, Utsunomiya K, Koya D, Babazono T, Moriya T, Makino H, Kimura K, Suzuki Y, Wada T, Ogawa S, Inaba M, Kanno Y, Shigematsu T, Masakane I, Tsuchiya K, Honda K, Ichikawa K, Shide K; Joint Committee on Diabetic Nephropathy: A new Classification of Diabetic Nephropathy 2014: A report from Joint Committee on Diabetic Nephropathy. J Diabetes Investig, 2015; 6: 242-246
17) Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E, Ito C, Inagaki N, Iwamoto Y, Kasuga M, Hanafusa T, Haneda M, Ueki K: Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig, 2010; 1: 212-228
18) Holick MF: Vitamin D deficiency. N Engl J Med, 2007; 357: 266-281
19) Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A: Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol, 2004; 44: 2137-2141
20) Syvänen K, Korhonen P, Partanen A, Aarnio P: Endothelial function in a cardiovascular risk population with borderline ankle-brachial index. Vasc Health Risk Manag, 2011; 7: 97-101
21) Igari K, Kudo T, Toyofuku T, Inoue Y: The Relationship between Endothelial Dysfunction and Endothelial Cell Markers in Peripheral Arterial Disease. PLoS one, 2016; 11: e0166840
22) Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, Mitchell GF, Sheffy J, Vita JA, Benjamin EJ: Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation, 2008; 117: 2467-2474
23) Hamburg NM, Palmisano J, Larson MG, Sullivan LM, Lehman BT, Vasan RS, Levy D, Mitchell GF, Vita JA, Benjamin EJ: Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. Hypertension, 2011; 57: 390-396
24) Dalan R, Liew H, Assam PN, Chan ES, Siddiqui FJ, Tan AW, Chew DE, Boehm BO, Leow MK: A randomised controlled trial evaluating the impact of targeted vitamin D supplementation on endothelial function in type 2 diabetes mellitus: The DIMENSION trial. Diab Vasc Dis Res, 2016; 13: 192-200
25) Reynolds JA, Haque S, Williamson K, Ray DW, Alexander MY, Bruce IN: Vitamin D improves endothelial dysfunction and restores myeloid angiogenic cell function via reduced CXCL-10 expression in systemic lupus erythematosus. Sci Rep, 2016; 6: 22341
26) Mangge H, Weghuber D, Prassl R, Haara A, Schnedl W, Postolache TT, Fuchs D: The role of vitamin D in atherosclerosis inflammation revisited: More a bystander than a player? Curr Vasc Pharmacol, 2015; 13: 392-398
27) Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R, Laakso M, Topliss D, Jenkins AJ, Blankenberg S, Burton A, Keech AC; FIELD Study Investigators: Serum 25-hydroxyvitamin D: A predictor of macrovascular and microvascular complications in patients with type 2 diabetes. Diabetes care, 2015; 38: 521-528
28) Joergensen C, Gall MA, Schmedes A, Tarnow L, Parving HH, Rossing P: Vitamin D levels and mortality in type 2 diabetes. Diabetes care, 2010; 33: 2238-2243
29) Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD: Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. Diabet Med, 2008; 25: 320-325
30) Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Lau CP, Cheung BM, Tse HF: Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. Atherosclerosis, 2013; 227: 140-146
31) Barchetta I, Del Ben M, Angelico F, Di Martino M, Fraioli A, La Torre G, Saulle R, Perri L, Morini S, Tiberti C, Bertoccini L, Cimini FA, Panimolle F, Catalano C, Baroni MG, Cavallo MG: No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. BMC Med, 2016; 14: 92
32) Pincombe NL, Pearson MJ, Smart NA, King N, Dieberg G: Effect of vitamin D supplementation on endothelial function - An updated systematic review with meta-analysis and meta-regression. Nutrition, metabolism, and cardiovascular diseases. Nutr Metab Cardiovasc Dis, 2019; 29: 1261-1272
33) LeBlanc ES, Pratley RE, Dawson-Hughes B, Staten MA, Sheehan PR, Lewis MR, Peters A, Kim SH, Chatterjee R, Aroda VR, Chadha C, Neff LM, Brodsky IG, Rosen C, Desouza CV, Foreyt JP, Hsia DS, Johnson KC, Raskin P, Kashyap SR, O'Neil P, Phillips LS, Rasouli N, Liao EP, Robbins DC, Pittas AG; D2d Research Group: Baseline characteristics of the vitamin D and type 2 diabetes (D2d) study: A contemporary prediabetes cohort that will inform diabetes prevention efforts. Diabetes Care, 2018; 41: 1590-1599