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Association of vitamin D deficiency with insulin resistance in middle-aged type 2 diabetics

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ABSTRACT

Background: Vitamin D deficiency contributes to the pathophysiology of insulin resistance (IR) and type 2 diabetes mellitus (T2DM). We investigated the association of 25-hydroxyvitamin D [25(OH)D] with IR and β-cell function in middle-aged participants.

Methods: We enrolled 90 controls and 90 T2DM patients of both genders aged 30-50 years. Serum 25(OH)D, fasting plasma insulin (FPI), fasting plasma glucose (FPG), HbA1c, and lipid profile were measured by standard methods. Insulin resistance and sensitivity were assessed by triglyceride glucose (TyG) index, homeostatic model assessment (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and β-cell function by HOMA-B.

Results: 25(OH)D deficiency was reported as 40% in control and 70% in T2DM patients. 25(OH)D concentration was positively associated with age, blood pressure, T2DM duration, FPG, HbA1c, TyG index, and HOMA-IR and negatively associated with HOMA-B and QUICKI among all the participants ($p \leq .001$). Participants with severe 25(OH)D deficiency (< 10 ng/ml) were 39 times higher odds of being T2DM, while, those with moderate deficiency (10-19ng/ml) and insufficiency (20–29 ng/ml) were 16 times and 13 times higher odds of being T2DM, respectively.

Conclusion: Sufficient 25(OH)D concentration may lower the risk of development of IR and T2DM in middle-aged control and diabetic participants.

1. Introduction

Over the past decades, the crucial role of vitamin D has been reported in many non-skeletal diseases including T2DM. Vitamin D deficiency or insufficiency is a significant risk factor for the development of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [1]. Vitamin D exists in two forms, i.e., ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Ergocalciferol is obtained from the plant sources while cholecalciferol is synthesized under the epidermis on exposure to the sunlight and also obtained from dietary sources such as fatty fish, fortified foods, and a supplement [2]. Vitamin D2 and D3 hydroxylated in the liver and converted into 25-hydroxyvitamin D [25(OH)D2 and 25(OH)D3], a major circulating metabolite of vitamin D. It reflects both vitamin D intake and endogenous production and is used to assess vitamin D status [3]. In the kidney, 25(OH)D hydroxylated to its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D2 and 1,25(OH)2D3] and these active metabolites bind to the vitamin D receptors and exert its biological activity [2].

The protective role of vitamin D in T2DM is speculated due to its effect on calcium metabolism, stimulation of insulin receptor gene, increase in calcium concentration in the cells, and enhancing glucose uptake into the muscle [4,5]. The active form of vitamin D, i.e., 1,25(OH)2D improves insulin sensitivity (IS) of insulin-target tissues through regulation of nuclear PPAR (peroxisome proliferative activated receptor) [6] and enhances the biosynthetic capacity of β-cells and expedites the conversion of proinsulin to insulin [7]. It indirectly improves IS by reducing adiposity and enhancing muscle mass [8].

Several studies have reported the association of 25(OH)D with IR and β-cell function in prediabetic and T2DM patients [1,9,10]. This association was also reported among healthy and glucose tolerant

Abbreviations: T2DM, type 2 diabetes mellitus; IR, insulin resistance; IS, insulin sensitivity; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; WHtR, waist to height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FPG, Fasting plasma glucose; FPI, Fasting plasma insulin; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, homeostatic model assessment for assessing β-cell function; QUICKI, Quantitative insulin sensitivity check index; ρ, Spearman's rank correlation coefficient; B, Unstandardized coefficient; β, Standardized coefficient

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subjects [7]. However, the underlying mechanism through which 25(OH)D affects the development and progression of IR and T2DM is not clearly understood in the middle-aged Indian subjects. Such an association needs further exploration in population-based studies, as the relationship of vitamin D metabolites with diabetes differs in different racial groups [11].

2. Materials and methods

2.1. Study design and population

A case-control study was performed at Symbiosis School of Biological Sciences (SSBS), Symbiosis International (Deemed University), Pune (India). Fasting blood samples of 180 male and female participants aged 30–50 y were collected from March 2016 to February 2018 and distributed into 2 groups as controls (n = 90) and T2DM patients (n = 90). All the samples were randomly collected at local region and diabetic clinics from Pune (India).

2.2. Study area

The study was conducted at Pune, a metropolitan city of Maharashtra State (India). Pune city is within Pune district and located between 18°32' North latitude and 72°51′ East longitude. It is at an altitude of 560 m above mean sea level.

2.3. Ethics statement

The study was conducted following the principles enshrined under the 'Declaration of Helsinki' and was approved by the Independent Ethics Committee (Institutional Ethics Committee) of Symbiosis International (Deemed University), Pune (India). Written informed consent was obtained from each participant prior to their inclusion in the study.

2.4. Inclusion and exclusion criteria

The control group comprised of apparently healthy participants having no prior history of T2DM and hypertension, and not receiving any medications at the time of sample collection. T2DM patients with a proven history of T2DM and receiving oral hypoglycemic drugs or insulin, but no other complications were included in the T2DM group. The exclusion criteria for both control and T2DM groups defined as pregnant and lactating women, subjects on vitamin D or calcium supplementation, subjects with liver diseases, renal diseases, cancer, pigment disorders, and other chronic medical illness.

2.5. Measurements

2.5.1. Preliminary data

The information such as age, gender, dietary habits, marital status, medical history (personal and family), lifestyle-related information, addictive habits, physical exercise, education level, occupation, and family income per month was collected by using questionnaire. Addictive habits were divided into two categories; viz., alcohol consumption and smoking (users of all types of tobacco products considered as smokers). Socioeconomic status was assessed by using Kuppuswamy's socioeconomic status scale updated for 2017 [12].

2.5.2. Anthropometric and blood pressure measurements

Body weight and height were measured using a digital scale and stadiometer, respectively and body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters. Waist circumference (WC) and hip circumference (HC) were measured using a non-stretchable measuring tape. WC was measured at the approximate midpoint between the lower margin of the last palpable rib and top of the iliac crest. HC was measured at levels of the greater trochanter. Systolic and diastolic blood pressure (SBP and DBP) of all participants were measured with a validated automatic digital blood pressure monitor (Omron Hem8712) and mean arterial blood pressure (MAP) was computed as [13], $MAP = P_{diastolic} + 1/3(P_{systolic}\text{-}P_{diastolic})$. Hypertension was defined as SBP > 140 mmHg and DBP > 90 mmHg or the subject undergoing antihypertensive treatment.

2.6. Sample collection

The blood sample was collected from the antecubital vein after 10–12 h of fasting and dispensed into two separate vacutainers (Becton Dickinson) containing K_2-EDTA and clot activator. Samples were centrifuged at 1500 × g for 10 min, plasma and serum were separated, aliquoted in cryovials, and stored at −80 °C. The subsequent analysis of biochemical variables was completed within one month, from the date of sample collection.

2.7. Biochemical assays

Hemoglobin A1c (HbA1c) measured as a mean of blood glucose concentration over the past 2–3 months by using the latex agglutination inhibition assay using Randox reagent kit. Fasting plasma glucose (FPG) was measured by glucose oxidase-peroxidase (GOD-POD) end-point assay using Span Diagnostics reagent kits. Triglycerides (TG), total cholesterol (TC), and high-density lipoproteins cholesterol (HDLc) were measured by means of glycerol-3-phosphate oxidase (GPO)-peroxidase 4-aminoantipyrine (PAP), cholesterol oxidase (CHOD)-PAP, and polyethylene glycol (PEG)-CHOD-PAP end-point enzymatic assays, respectively using Span Diagnostics reagent kits. Low-density lipoprotein cholesterol (LDLc) estimated by using the Friedewald formula [14]. Triglyceride glucose (TyG) index [15], a simple measure of insulin resistance and apolipoprotein B (Apo B) [16] determined as follows:

$$\text{TyG index} = \ln[\text{TG (mg/dl)} \times \text{FPG (mg/dl)}/2]$$

$$\text{ApoB} = [-33.12 + 0.675 \times \text{LDLc} + 11.95 \times \ln(\text{TG})]$$

Serum total 25(OH)D [25(OH)D_2 and 25(OH)D_3] was determined by using a direct quantitative enzyme immunoassay (Diasource, Belgium), in which the biotin-labeled 25(OH)D competes with the 25(OH)D present in the calibrators, controls, and samples for the binding sites on the monoclonal antibodies. Horseradish peroxidase with tetramethylbenzidine used as a substrate for color development. The absorbance was measured at 450 nm by a microplate reader. A standard curve was constructed, and the 25(OH)D concentration in the samples were calculated. The analytical measurement range of this assay was 0 to 105 ng/ml. The cross-reactivity of the assay kit was 100% with 25(OH)D3 and 79.8% with 25(OH)D2. The coefficients of variation for intra-assay precision of the assay kit were 3.6% and 8.6% at mean 25(OH)D concentration of 21.8 ± 0.8 and 47.7 ± 4.1 ng/ml, respectively and inter-assay precision were 6.4% and 7.7% at mean 25(OH)D concentration of 17.7 ± 1.1 and 38.5 ± 3.0 ng/ml, respectively. Serum 25(OH)D concentration were stratified into clinically relevant categories as severe deficiency (< 10 ng/ml), moderate deficiency (10–19 ng/ml), insufficiency (20–29 ng/ml), and sufficiency (> 30 ng/ml) [17].

A direct quantitative enzyme immunoassay (Invitrogen, USA) was used to determine fasting plasma insulin, in which anti-insulin antibody-coated wells were incubated with anti-insulin horseradish peroxidase conjugate and standards or controls or samples. Tetramethylbenzidine and hydrogen peroxide (H_2O_2) used as a substrate for color development and the absorbance was measured at 450 nm by a microplate reader. A curve was constructed, and the concentration of insulin in the samples were calculated. The analytical sensitivity of this assay was 0.17μIU/ml of human insulin. The coefficients of variation (CVs) for intra-assay precision of the assay kit were

4.8% and 6% at mean human insulin concentration of 13.09 ± 0.6 and 32.9 ± 1.9 μIU/ml, respectively and inter-assay CVs were 8.1% and 9% at mean human insulin concentration of 13.29 ± 1.08 and 34.12 ± 3.1 μIU/ml, respectively. IR and IS were assessed by homeostatic model assessment index (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI), respectively and β-cell function by HOMA-B by using the following formulae [18]:

$$\text{HOMA} - \text{IR} = \{[\text{fasting insulin (uIU/ml)}] \times [\text{fasting glucose (mmol/l)}]\} / 22.5$$

$$\text{HOMA} - \text{B\%} = [20 \times \text{fasting insulin (uIU/ml)}] / [\text{fasting glucose (mmol/l)} - 3.5]$$

$$\text{QUICKI} = 1/[\log(\text{fasting insulin, uIU/ml}) + \log(\text{fasting glucose, mg/dl})]$$

2.8. Statistical analyses

All the analyses were performed using SPSS software (ver. 16.0). The Kolmogorov-Smirnov test was used to determine the normality of variables. Categorical data were expressed as frequency counts (%). Variables following a normal distribution were expressed as mean and standard deviation (SD), and non-normal distribution as median with interquartile ranges (IQR). The means of variables were compared using the chi-square test for categorical data, Student's *t*-test for normally distributed variables, and Mann-Whitney *U* test for non-normally distributed variables. To establish correlations between variables the Spearman rank order correlation was used. Linear regression analysis was used to test the relationship between the independent variables and a dependent variable. A $p < .05$ was considered as statistically significant.

3. Results

3.1. Socio-demographic characteristics, anthropometric, and biochemical variables in the control and T2DM groups

The socio-demographic characteristics of the controls and T2DM patients are shown in Table 1 and indicate that there were more male (57.8% in control and 55.6% in T2DM group) than female (42.2% in control and 44.4% in T2DM group) participants in each group. Also, more sujects were married (88.9% in control and 97.8% in T2DM group) than non-married (11.1% in control and 2.2% in T2DM group). Less number of participants were vegetarian (26.7% in control and 43.3% in T2DM group) than non-vegetarian (73.3% in control and 56.7% in T2DM group). Less number of participants have a history of smoking and alcohol intake in both groups. In control group 39.3% and T2DM group 88.9% participants have the family history of diabetes (Table 1).

The anthropometric and biochemical variables of the control and T2DM groups are shown in Table 2 and indicate that BMI, WC, HC, WHtR, SBP, DBP, MAP, TG, VLDLc, FPG, and HbA1c were significantly higher in T2DM patients as compared to the control participants ($p \leq .001$) (Table 2). The mean ± SD, median (Q1-Q3), minimum, and maximum values for 25(OH)D, IR, and IS indices of the controls and T2DM patients are shown in Table 3 and indicate that 25(OH)D and all studied IR and IS indices (TyG index, FPI, HOMA-IR, HOMA-B, and QUICKI) were highly significant in control and T2DM groups (Table 3).

The socio-demographic, clinical, anthropometric, and biochemical variables among control and T2DM participants stratified by 25(OH)D concentration (severe deficiency ≤ 10 ng/ml, moderate deficiency = 10–19 ng/ml, insufficiency = 20–29 ng/ml, and sufficiency ≥ 30 ng/ml) are shown in Table 4A and 4B. The Student's *t*-test was applied to compare means of all variables and data were expressed as mean ± SD. In severe deficient, moderate deficient, and insufficient

Table 1
Socio-demographic characteristics of control and T2DM participants.

Variables	Control (n = 90)	T2DM (n = 90)	(χ^2, df) p value
Gender			(0.764,1) NS
Male	52 (57.8%)	50 (55.6%)	
Female	38 (42.2%)	40 (44.4%)	
Marital Status			**(5.714, 1) 0.032**
Unmarried	10 (11.1%)	2 (2.2%)	
Married	80 (88.9%)	88 (97.8%)	
Diet (%)			**(5.495, 1) 0.028**
Vegetarian	24 (26.7%)	39 (43.3%)	
Non- vegetarian	66 (73.3%)	51 (56.7%)	
Smoking (%)			**(5.714, 1) 0.032**
Yes	2 (2.2%)	10 (11.1%)	
No	88 (97.8%)	80 (88.9%)	
Alcohol intake (%)			(0.988, 1) NS
Yes	7 (7.8%)	11 (12.2%)	
No	83 (92.2%)	79 (87.8%)	
Physical exercise (%)			**(13.117, 1)**
Yes	40 (44.4%)	64 (71.1%)	**< 0.001**
No	50 (55.6%)	26 (28.9%)	
Family History of T2DM (%)			**(47.855, 1) < 0.001**
Yes	35 (39.3%)	80 (88.9%)	
No	55 (60.7%)	10 (11.1%)	
Socioeconomic Status			(11.013, 3) NS
Upper	32 (35.6%)	14 (15.6%)	
Upper middle	32 (35.6%)	41 (45.6%)	
Lower middle	18 (20%)	29 (32.1%)	
Upper lower	8 (8.8%)	6 (6.7%)	

All categorical variables were presented in frequency counts (percentage). Significant p values were shown in bold. χ^2: Chi-square test, df:degree of freedom.

Table 2
Anthropometric and biochemical variables of control and T2DM groups.

Variables	Control (n = 90)	T2DM (n = 90)	p-Value
Age (y)	37.97 ± 6.14	41.83 ± 5.91	**< 0.001**
Anthropometric parameters			
Weight (Kg)	68.09 (58.53–78.25)	70.45 (63.68–77.73)	NS
Height (cm)	163.45 ± 10.32	160.47 ± 9.05	**0.018**
BMI (Kg/m^2)	25.59 ± 3.95	28.46 ± 3.68	**< 0.001**
WC (cm)	91.23 (85.44–97.09)	95.45 (90.88–100.25)	**0.001**
HC (cm)	98.25 (93.22–103.12)	101.45 (98.45–156.4)	**< 0.001**
WHR	0.94 (0.90–0.97)	0.94 (0.89–0.97)	NS
WHtR	0.56 (0.52–0.60)	0.60 (0.56–0.63)	**< 0.001**
Blood pressure			
SBP (mmHg)	120.45 (113.32–125.54)	125.55 (120.45–135.67)	**< 0.001**
DBP (mmHg)	80.14 (74.38–80.35)	80.35 (80.34–85.76)	**< 0.001**
MAP (mmHg)	92.92 (87.25–95.25)	96.67 (93.33–100.09)	**< 0.001**
Hypertension (%)	0 (0%)	30 (33.33%)	–
T2DM duration (years):	–	5.23 ± 3.79	–
Biochemical Indices			
TG (mmol/l)	1.13 (0.93–1.55)	1.41 (0.99–1.98)	**0.004**
TC (mmol/l)	3.94 ± 0.84	3.91 ± 0.93	NS
HDLc (mmol/l)	0.99 (0.89–1.25)	0.95 (0.85–1.13)	NS
LDLc (mmol/l)	2.29 ± 0.79	2.19 ± 0.85	NS
VLDLc (mmol/l)	0.52 (0.43–0.71)	0.65 (0.45–0.91)	**0.004**
Apo-B (mg/dl)	82.41 ± 21.54	81.94 ± 23.06	NS
FPG (mg/dl)	82.09 (75.97–89.73)	129.42 (110.54–165.23)	**< 0.001**
HbA1c (%)	5.26 (4.91–5.53)	7.85 (6.82–8.81)	**< 0.001**

Data are mean ± SD and median (Q1–Q3) for normally and non-normally distributed variables, respectively. Significant values were shown in bold.
Abbreviations: WC: waist circumference; HC: hip circumference; WHR: waist to hip ratio; WHtR: waist to height ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; FPG: Fasting plasma glucose, NS: non-significant.

Table 3
25(OH)D, insulin resistance and sensitivity indices in control and T2DM groups.

Variables	Group	Mean ± SD	Median (IQR)	Minimum	Maximum	p value
*25(OH)D (ng/ml)	Control	23.92 ± 9.93	23.35 (16.21–30.91)	6.04	50.51	< **0.001**
	T2DM	16.68 ± 7.51	16.77 (9.93–22.43)	3.72	41.22	
*TyG index	Control	8.40 ± 0.44	8.32 (8.08–8.65)	7.66	9.92	< **0.001**
	T2DM	9.07 ± 0.58	9.03 (8.63–9.46)	8.07	11.01	
*FPI (uIU/ml)	Control	16.72 ± 6.23	16.76 (10.95–22.34)	4.22	29.02	**0.004**
	T2DM	22.43 ± 17.29	17.91 (12.82–27.33)	4.83	129.48	
*HOMA-IR	Control	3.45 ± 1.39	3.47 (2.26–4.43)	0.95	8.22	< **0.001**
	T2DM	7.10 ± 8.07	6.09 (3.88–9.55)	1.53	68.67	
*HOMA-B	Control	418.22 ± 359.22	302.62 (194.43–485.82)	71.63	3348.48	< **0.001**
	T2DM	130.51 ± 112.17	97.15 (53.72–177.09)	11.76	662.78	
**QUICKI	Control	0.32 ± 0.02	0.32 (0.31–0.34)	0.28	0.39	< **0.001**
	T2DM	0.30 ± 0.03	0.30 (0.28–0.31)	0.23	0.36	

SD: Standard deviation; IQR: Interquartile ranges; 25(OH)D: 25 hydroxyvitamin D; TyG index: Triglyceride glucose index; FPI: Fasting plasma insulin; HOMA-IR: homeostatic model assessment for insulin resistance; HOMA-B: homeostatic model assessment for assessing β-cell function; QUICKI: Quantitative insulin sensitivity check index.

* Mann-Whitney *U* test was used.

** Student's *t*-test was used.

categories, SBP, MAP, FPG, HbA1c, TyG index, HOMA-IR, HOMA-B, and QUICKI were significantly different in the control and T2DM groups. While, in sufficient category weight, BMI, FPG, HbA1c, and HOMA-B showed a significant difference between the control and T2DM groups. Also, in severe deficient and moderate deficient categories BMI, WC, and WHtR were significantly different for the control and T2DM groups (Tables 4A and 4B].

3.2. Prevalence of different categories of 25(OH)D in the control and T2DM groups

The prevalence of different categories of 25(OH)D in the control and T2DM groups are shown in Table 5. The prevalence of severe and moderate 25(OH)D deficiency was 28.9% and 41.1%, respectively in T2DM participants and 10% and 30%, respectively in controls. In the control group, 31.1% and in T2DM group 3.3% participants were having sufficient concentration of 25(OH)D. Logistic regression analysis revealed that participants with severe 25(OH)D deficiency were 39 times higher odds of being T2DM, while, participants with moderate deficiency and insufficiency were 16 times and 13 times higher odds of being T2DM, respectively, after adjusted to age, BMI, and gender (Table 5).

3.3. Correlation and linear regression analysis among all the participants

Correlation and linear regression analysis of 25(OH)D (dependent variable) with various anthropometric and biochemical variables (independent variables) among all the participants are presented in Table 6. This analysis was done by using Spearman's rank order correlation and linear regression. In linear regression analysis, unstandardized coefficient indicates the change in dependent variable [25(OH)D] with a unit increment in the independent variable, while the

Table 4A
Anthropometric indices, blood pressure, and biochemical variables in control and T2DM groups stratified by 25(OH)D concentration.

Variables	Severe deficient (< 10 ng/ml)		p value	Moderate deficient (10-20 ng/ml)		p value
	Control	T2DM		Control	T2DM	
Number	9	26	–	27	37	–
Age (y)	38.89 ± 4.46	42.54 ± 5.36	NS	40.19 ± 6.45	42.03 ± 6.44	NS
Weight (kg)	62.79 ± 13.91	72.43 ± 10.82	0.037	66.23 ± 12.28	69.98 ± 10.96	NS
Height (cm)	165.57 ± 6.22	160.22 ± 8.53	NS	160.64 ± 11.24	158.68 ± 9.21	NS
BMI (Kg/m^2)	22.87 ± 4.46	28.18 ± 3.33	0.001	25.65 ± 3.93	27.79 ± 3.62	0.028
WC (cm)	86.22 ± 8.91	96.83 ± 9.05	0.002	88.72 ± 11.39	94.65 ± 7.24	0.014
HC (cm)	92.56 ± 9.91	103.52 ± 5.91	0.011	97.19 ± 12.90	101.46 ± 6.66	NS
WHR	0.93 ± 0.03	0.94 ± 0.13	NS	0.92 ± 0.08	0.93 ± 0.06	NS
WHtR	0.52 ± 0.05	0.61 ± 0.07	0.002	0.55 ± 0.07	0.60 ± 0.05	0.005
SBP (mmHg)	120.24 ± 7.22	133.83 ± 15.33	0.019	120.76 ± 9.89	127.77 ± 11.98	0.016
DBP (mmHg)	80.15 ± 2.53	85.89 ± 7.42	0.002	78.50 ± 9.04	81.45 ± 5.06	NS
MAP (mmHg)	93.48 ± 3.74	101.86 ± 9.32	0.001	92.59 ± 8.64	96.89 ± 6.64	0.036
TG (mmol/l)	1.18 ± 0.42	1.65 ± 0.62	NS	1.30 ± 0.47	1.48 ± 0.72	NS
TC (mmol/l)	3.98 ± 1.09	4.11 ± 0.91	NS	4.05 ± 0.98	3.64 ± 0.93	NS
HDLc (mmol/l)	1.08 ± 0.22	0.98 ± 0.19	NS	1.02 ± 0.18	1.01 ± 0.29	NS
LDLc (mmol/l)	2.35 ± 1.22	2.37 ± 0.82	NS	2.44 ± 0.87	1.96 ± 0.82	0.027
VLDLc (mmol/l)	0.54 ± 0.24	0.76 ± 0.31	NS	0.60 ± 0.21	0.68 ± 0.33	NS
Apo-B (mg/dl)	83.14 ± 32.77	87.62 ± 21.44	NS	86.52 ± 24.36	74.96 ± 22.90	NS
FPG (mg/dL)	83.74 ± 9.56	154.83 ± 48.42	< 0.001	82.47 ± 9.47	132.33 ± 36.36	< 0.001
HbA1c (%)	5.22 ± 0.45	8.23 ± 1.32	< 0.001	5.09 ± 0.47	7.85 ± 1.17	< 0.001
TyG index	8.34 ± 0.43	9.23 ± 0.54	< 0.001	8.40 ± 0.38	8.93 ± 0.54	< 0.001
FPI (uIU/ml)	15.35 ± 6.76	23.83 ± 24.45	NS	17.10 ± 6.33	23.71 ± 14.36	0.016
HOMA-IR	3.08 ± 1.23	9.62 ± 13.03	0.042	3.52 ± 1.45	7.67 ± 5.08	< 0.001
HOMA-B	461.91 ± 515.62	116.52 ± 107.44	0.004	388.35 ± 261.53	158.63 ± 129.24	< 0.001
QUICKI	0.33 ± 0.02	0.30 ± 0.03	0.011	0.32 ± 0.02	0.29 ± 0.02	< 0.001

Table 4B
Anthropometric indices, blood pressure, and biochemical variables in control and T2DM groups stratified by 25(OH)D concentration.

Variables	Insufficient (20-30 ng/ml)		p value	Sufficient (≥30 ng/ml)		p value
	Control	T2DM		Control	T2DM	
Number	26	24	–	28	3	–
Age (y)	36.00 ± 5.18	40.79 ± 5.82	**0.003**	37.36 ± 6.73	41.67 ± 4.22	NS
Weight (Kg)	70.23 ± 13.88	71.83 ± 14.69	NS	70.73 ± 11.22	90.67 ± 9.51	**0.005**
Height (cm)	164.44 ± 10.10	160.98 ± 9.72	NS	164.62 ± 10.62	166.67 ± 4.22	NS
BMI (kg/m^2)	25.86 ± 3.66	27.61 ± 3.88	NS	26.19 ± 3.91	32.78 ± 4.92	**0.010**
WC (cm)	92.81 ± 8.18	93.58 ± 10.70	NS	92.68 ± 7.42	101.12 ± 11.11	NS
HC (cm)	99.08 ± 7.30	100.60 ± 10.42	NS	98.89 ± 8.24	110.23 ± 20.22	NS
WHR	0.94 ± 0.05	0.94 ± 0.11	NS	0.94 ± 0.11	0.92 ± 0.12	NS
WHtR	0.57 ± 0.05	0.58 ± 0.06	NS	0.57 ± 0.08	0.61 ± 0.09	NS
SBP (mmHg)	118.73 ± 10.29	128.23 ± 9.79	**0.002**	117.86 ± 8.82	121.32 ± 8.11	NS
DBP (mmHg)	77.92 ± 7.13	81.69 ± 7.99	NS	77.83 ± 6.44	80.33 ± 1.22	NS
MAP (mmHg)	91.53 ± 7.18	97.20 ± 7.35	**0.008**	91.17 ± 6.75	93.78 ± 2.73	NS
TG (mmol/l)	1.35 ± 0.78	1.73 ± 1.07	NS	1.38 ± 1.03	1.26 ± 0.45	NS
TC (mmol/l)	3.97 ± 0.65	4.13 ± 0.93	NS	3.79 ± 0.81	4.15 ± 0.73	NS
HDLc (mmol/l)	1.08 ± 0.21	1.02 ± 0.23	NS	1.02 ± 0.24	1.03 ± 0.12	NS
LDLc (mmol/l)	2.28 ± 0.67	2.31 ± 0.93	NS	2.14 ± 0.76	2.54 ± 0.65	NS
VLDLc (mmol/l)	0.62 ± 0.36	0.79 ± 0.49	NS	0.63 ± 0.44	0.58 ± 0.22	NS
Apo-B (mg/dl)	82.30 ± 16.93	85.68 ± 23.91	NS	78.33 ± 18.42	88.96 ± 19.44	NS
FPG (mg/dl)	83.18 ± 9.10	148.53 ± 49.10	< **0.001**	84.23 ± 12.33	114.73 ± 13.74	< **0.001**
HbA1c (%)	5.10 ± 0.42	7.92 ± 1.18	< **0.001**	5.47 ± 0.44	7.04 ± 0.55	< **0.001**
TyG index	8.41 ± 0.43	9.16 ± 0.69	< **0.001**	8.43 ± 0.54	8.73 ± 0.46	NS
FPI (uIU/ml)	14.15 ± 5.70	19.37 ± 12.33	NS	19.14 ± 5.75	19.39 ± 11.91	NS
HOMA-IR	2.90 ± 1.17	7.05 ± 4.63	< **0.001**	4.23 ± 1.44	5.74 ± 3.92	NS
HOMA-B	452.96 ± 661.47	102.53 ± 85.30	**0.013**	489.22 ± 422.42	129.49 ± 55.33	< **0.001**
QUICKI	0.33 ± 0.02	0.30 ± 0.03	< **0.001**	0.32 ± 0.02	0.31 ± 0.03	NS

Bold signifies the p value < 0.05 were considered as statistically significant.

standardized coefficient shows the impact of independent variables on the dependent variable. A significant negative correlation of 25(OH)D with age, SBP, DBP, MAP, duration of T2DM, TG, FPG, HbA1c, TyG index, and HOMA-IR and a significant positive correlation with HOMA-B and QUICKI were observed among all the participants. Linear regression analysis revealed that 25(OH)D has a significant negative association with age, SBP, DBP, MAP, duration of T2DM, FPG, HbA1c, TyG index, and HOMA-IR, and the significant positive association with HOMA-B and QUICKI among all the participants (Table 6).

4. Discussion

In the present study, we found that 70% T2DM and 40% controls have a low concentration of 25(OH)D (severe and moderate deficiency). Previous studies have also reported the high prevalence of 25(OH)D deficiency in Indian T2DM and healthy subjects, despite the fact that, India is exposed to ample sunlight throughout the year [19–21]. A prospective cross-sectional study from Western India has reported a high prevalence of 25(OH)D deficiency as 91.4% in T2DM and 93% in control participants [20]. In another cross-sectional study from South India, 25(OH)D deficiency was observed as 44% and 62% in rural and urban men, respectively, while, 70% and 75% in rural and urban women, respectively [21]. The 25(OH)D deficiency is influenced by many factors such as age (> 80 y), BMI (> 30 kg/m^2), race (non-white), use of medications those affect vitamin D metabolism, lack of physical exercise, lifestyle, and underlying health conditions (common cancers, cardiovascular diseases, multiple sclerosis, and autoimmune diseases) [22–25].

The major circulating form of vitamin D, i.e., 25(OH)D is known to be stored in adipose tissues. Obese individuals tend to have lower 25(OH)D concentration due to excess adiposity, as the 25(OH)D accumulates in the adipose tissue [26,27]. In the present study, we found the significant increase in the means of TyG index, FPI, and HOMA-IR in the T2DM group, and HOMA-B, QUICKI, and 25(OH)D in the control group. We also found that glycemic markers (FPG and HbA1c) and adiposity measures including BMI, WC, HC, WHtR, TG, and VLDLc were significantly higher in T2DM patients as compared to the control participants.

Further, we stratified the control and T2DM groups in clinically relevant categories of 25(OH)D concentration. It shows that, in both severe deficiency and insufficiency category, means of BMI, WHtR, BP, glycemic markers (FPG, HbA1c), IR and IS indices (TyG index, HOMA-IR, HOMA-B, and QUICKI) were significantly different in the control and T2DM groups. While, in sufficiency category means of weight, BMI, FPG, HbA1c, and HOMA-B were significantly different in control and T2DM groups. In addition, the logistics regression analyses were performed to confirm the causal relationship between 25(OH)D concentration and T2DM. The regression analysis revealed that the participants with severe 25(OH)D deficiency were 39 times higher odds of being T2DM. While, those with moderate deficiency and insufficiency were 16 times and 13 times higher odds of being T2DM, respectively.

In the present study, correlation analysis indicates that 25(OH)D has a significant negative association with age, BP, T2DM duration, FPG, HbA1c, TyG index, and HOMA-IR, and a significant positive association

Table 5
Prevalence and odds of different categories of 25(OH)D among control and T2DM groups.

Categories	Control (n = 90)	T2DM (n = 90)	OR (95% CI)	p value	OR (95% CI) Age, BMI, gender adjusted	p value
Severe deficiency (< 10 ng/ml)	9 (10%)	26 (28.9%)	26.963 (6.573–110.602)	< 0.001	38.927 (7.985–189.778)	< 0.001
Moderate deficiency (10–19 ng/ml)	27 (30%)	37 (41.1%)	12.790 (3.521–46.458)	< 0.001	15.716 (3.806–64.895)	< 0.001
Insufficiency (20–29 ng/ml)	26 (28.9%)	24 (26.7%)	8.615 (2.316–32.045)	0.001	13.359 (3.137–56.890)	< 0.001
Sufficiency (> 30 ng/ml)	28 (31.1%)	3 (3.3%)	1.0 (reference)	–	1.0 (reference)	–

Table 6
Spearman's rank order correlation and linear regression analysis between 25 (OH) D concentrations and various anthropometric and biochemical variables in all participants.

Variables	25 (OH)D (ng/ml)					
	Correlation		Linear regression			
	ρ	p value	B	Std. Err.	β	p value
Age (y)	−0.241	0.001	−0.325	0.110	−0.216	0.004
BMI (Kg/m^2)	−0.022	NS	0.015	0.178	0.006	NS
WC (cm)	−0.024	NS	−0.032	0.063	−0.038	NS
HC (cm)	−0.059	NS	−0.021	0.077	−0.021	NS
WHtR	−0.103	NS	−12.544	9.788	−0.096	NS
SBP (mmHg)	−0.305	< 0.001	−0.223	0.056	−0.287	< 0.001
DBP (mmHg)	−0.268	< 0.001	−0.307	0.094	−0.237	0.001
MAP (mmHg)	−0.300	< 0.001	−0.331	0.084	−0.285	< 0.001
T2DM duration (years):	−0.350	< 0.001	−0.713	0.183	−0.281	< 0.001
TG (mmol/l)	−0.149	0.046	−0.762	0.929	−0.061	NS
TC (mmol/l)	−0.108	NS	−0.997	0.805	−0.092	NS
HDLc (mmol/l)	0.062	NS	0.551	3.189	0.013	NS
LDLc (mmol/l)	−0.084	NS	−0.888	0.867	−0.077	NS
Apo-B (mg/dl)	−0.119	NS	−0.041	0.032	−0.097	NS
FPG (mg/dl)	−0.324	< 0.001	−0.067	0.016	−0.306	< 0.001
HbA1c (%)	−0.311	< 0.001	−1.948	0.410	−0.335	< 0.001
TyG index	−0.272	< 0.001	−3.814	1.119	−0.248	0.001
FPI (uIU/ml)	−0.015	NS	−0.074	0.053	−0.104	NS
HOMA-IR	−0.148	0.047	−0.293	0.113	−0.192	0.010
HOMA-B	0.272	< 0.001	0.005	0.002	0.205	0.006
QUICKI	0.148	0.047	53.739	26.169	0.152	0.041

Significant values were shown in bold. ρ = Spearman's rank correlation coefficient; B: Unstandardized coefficient; Std. Err.: Standard Error; β: Standardized coefficient. Other abbreviations as in Table 2 & 3.
Bold signifies the p value < 0.05 were considered as statistically significant.

with HOMA-B and QUICKI among all the participants. However, no significant correlation of 25(OH)D with lipid profile and adiposity measures was found, except TG among all the participants. Vitamin D metabolites may modify the lipid profile through lipoprotein lipase activity in adiposity [28]. Also, vitamin D increases calcium absorption, which may reduce the formation and secretion of hepatic TG [29] and decreases the cholesterol level by promoting the secretion of bile acids [30]. Vitamin D plays a vital role in the regulation of glucose-insulin homeostasis. The active form of vitamin D [1,25(OH)$_2$D] binds to the vitamin D receptors (VDRs) present in β-cell and activates the VDR-retinoic acid X-receptor (RXR) complex. This complex binds to a vitamin D response element in the promoter region of the human insulin receptor gene and further enhances the insulin responsiveness for glucose transport. The potential effect of 1,25(OH)$_2$D on the improvement of IS perhaps due to its role in the regulation of calcium influx (through the cellular membrane in insulin target tissues) and stimulation of peroxisome proliferator-activated receptor delta (PPAR-δ), a transcription factor that mediates the fatty acids metabolism in skeletal muscle and adipose tissue [31,32].

Previous evidence on the association of 25(OH)D deficiency with IR, IS, and T2DM have been inconsistent, and many of them have found a significant association [7–11,33] while some reported null association [20,34,35]. Witham et al. and Kampmann et al. have shown that vitamin D supplementation may increase insulin secretion but did not improve IR and HbA1c in T2DM patients [36,37]. This might happen because inflammatory processes are already at their maximum in diabetic condition or the β-cell dysfunction and IR is more severe and less reversible with the longer duration of diabetes [34,38]. A better insulin secretory response after vitamin D supplementation was observed in newly diagnosed (within three years) T2DM patients [39]. Inflammation is considered as a key driver in the development of IR [40], and with the longer duration of T2DM, inflammation worsens the IR, suggesting that vitamin D supplementation may not be useful once β-cells are exhausted [41]. Thus, the role of vitamin D in the pathogenesis of IR, T2DM, and overall glucose homeostasis needs a detailed investigation.

The major outcome of our study highlights the association of 25(OH)D deficiency with IR in middle-aged subjects from the Western part of India. Several studies have been conducted in the geriatric population, those who already had 25(OH)D deficiency as older age decreases the UVB-induced cutaneous vitamin D synthesis [42]. To date, very few studies have been conducted in the middle-aged Indians. Thus, this study establishes the groundwork for future research that would explore the said association in the middle-aged population. There are notable limitations to our study. First, the present study has not investigated any cause and effect relationship. Second, the lack of use of gold standards such as euglycemic and hyperglycemic clamps to assess IR and third, it was a one point-single time study in a relatively small set of samples. Longitudinal data would be useful to determine how vitamin D repletion alters the development of IR and T2DM.

5. Conclusion

The present study highlights the high prevalence of 25(OH)D deficiency in Indian middle-aged T2DM patients as compared to control participants, despite the availability of ample sunlight throughout the year in most of the places. Due to increase in indoor lifestyles and use of sun avoidance strategies, 25(OH)D deficiency is alarmingly increasing in middle-aged diabetic and healthy subjects and is associated with glycemic markers, TG, IR and IS indices. Thus, our findings suggest that the optimal 25(OH)D concentration may prevent the development of IR and subsequent advancement in T2DM.

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Declarations of interest

Authors declared that they have no conflict of interest.

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