

Effect of Vitamin D Supplementation on Vascular Functions and Oxidative Stress in Type 2 Diabetic Patients with Vitamin D Deficiency

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Abstract

Background: Vitamin D levels are reported to have an inverse liaison with the risk of cardiovascular diseases. Hence, we aimed to evaluate the effect of Vitamin D supplementation on changes in vascular functions and oxidative stress in type 2 diabetic patients with Vitamin D deficiency. **Subjects and Methods:** One hundred and three patients with type 2 diabetes attending endocrinology outpatients department in a tertiary care hospital were screened for Vitamin D deficiency. Patients with serum 25-hydroxy Vitamin D levels <20 ng/ml were considered as deficient and were administered 60,000 IU of oral Vitamin D₃ weekly for 8 weeks. In these patients, parameters of vascular functions (carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, and arterial stiffness index) and oxidative stress (serum malondialdehyde levels and total antioxidant status) were measured at baseline and after 8 weeks of oral Vitamin D supplementation. **Results:** Among 103 patients with type 2 diabetes, 75 (72.82%) were found to have Vitamin D deficiency. Amidst these patients, carotid-femoral pulse wave velocity (991.6 ± 161.82 vs. 899.29 ± 151.86 , $P < 0.001$), right brachial-ankle pulse wave velocity (1446.16 ± 204.33 vs. 1350.8 ± 178.39 , $P < 0.001$), and left brachial-ankle pulse wave velocity (1493.81 ± 219.65 vs. 1367.61 ± 220.64 , $P < 0.001$) showed a significant reduction following Vitamin D supplementation. Further, these patients were found to have significant fall in serum malondialdehyde levels with rise in total antioxidant status ensuing Vitamin D supplementation. **Conclusion:** The present study shows that oral Vitamin D supplementation of 60,000 IU/week for 8 weeks significantly improves vascular functions and reduces oxidative stress in type 2 diabetic patients with Vitamin D deficiency.

Keywords: Arterial stiffness, oxidative stress, type 2 diabetes mellitus, Vitamin D deficiency

INTRODUCTION

Cardiovascular diseases constitute an imperative cause of morbidity and mortality in type 2 diabetes mellitus.^[1] Early identification of diabetic patients at risk for cardiovascular events and instigation of interventions to prevent progression of the disease process assumes high priority. Literature has shown increased arterial stiffness is associated with the occurrence of first cardiovascular event.^[2,3] Several indices such as carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, arterial stiffness index, central blood pressure, and augmentation index are used for the assessment of arterial stiffness.^[4] However, carotid-femoral pulse wave velocity is considered to be the gold standard index for the measurement of arterial stiffness.^[5]

Vitamin D deficiency is supposedly higher in type 2 diabetic patients compared to healthy individuals, and an inverse

relationship between Vitamin D levels and arterial stiffness has been demonstrated.^[6-9] It is postulated that Vitamin D improves endothelial function by reducing vascular inflammation, regulating blood pressure, inhibiting proliferation of vascular smooth muscle cells, and antagonizing the formation of foam cells.^[10] Further, antioxidant property of Vitamin D adds to protective mechanisms on heart and vasculature.^[10,11] Studies conducted so far to evaluate the effect of Vitamin D supplementation on arterial stiffness and other measures of vascular functions in Vitamin D deficient patients have shown

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inconsistent results.^[12-15] Hence, the present study was planned to evaluate the effect of oral Vitamin D supplementation on change in vascular functions (carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, and arterial stiffness index) and oxidative stress (serum malondialdehyde levels and total antioxidant status) in type 2 diabetic patients with Vitamin D deficiency.

SUBJECTS AND METHODS

The study was approved by Institutional Ethics Committee (Human Studies), JIPMER, Puducherry, and was registered in Clinical Trials Registry of India (CTRI/2016/03/006698). The study was conducted from May 2015 to June 2016 in accordance with the principles of Declaration of Helsinki. Patients diagnosed with type 2 diabetes mellitus for the last 10 years and on the stable treatment of metformin or sulfonylurea or both for the last 3 months in the age group of 35–60 years belonging to either gender attending endocrinology outpatients department, JIPMER, were included in the study. Written informed consent was obtained from all the eligible patients willing to participate in the study. The diabetic patients with liver dysfunction, renal dysfunction, concurrent illnesses other than hypertension, taking enzyme inducers or inhibitors or Vitamin D supplements, pregnant and lactating women, smokers and participated in other clinical studies within the last 3 months were excluded from the study.

Demographic characteristics of the enrolled patients were recorded, and 6 ml of venous blood was collected for analysis of serum 25-hydroxy Vitamin D (25-OH Vitamin D) (both D₂ and D₃), lipid profile, serum calcium, serum phosphorous, liver function, renal function, serum malondialdehyde, and total antioxidant status. Serum 25-OH Vitamin D levels were measured by chemiluminescence method; based on the level, patients were classified as Vitamin D deficient, insufficient, or normal. Vitamin D deficiency was defined as serum 25-OH Vitamin D level <20 ng/ml while insufficiency and normal levels were considered for serum 25-OH Vitamin D levels of 20–30 ng/ml and >30 ng/ml, respectively. Oral supplementation of 60,000 IU/week of cholecalciferol was given to patients with Vitamin D deficiency for a period of 8 weeks. The cholecalciferol sachets were procured through JIPMER pharmacy. Each sachet weighing 1 g contained 60,000 IU of cholecalciferol (ENumine D, Hexagon Group Co., Nasik). Initially, 4 sachets of cholecalciferol were given to patients and instructed to mix the contents of one sachet in 120 ml milk and drink it every week for a period of 4 weeks. All patients were given a chart and were requested to mention the date of intake of the drug as well as the occurrence of any adverse effects. During each follow-up visit, the participants were asked to return empty sachets of medication to ensure adherence. After 4 weeks of supplementation, 2 ml of venous blood was collected to measure their serum calcium and phosphorous levels, and another 4 sachets of cholecalciferol

granules containing 60,000 IU per sachet were given. After 8 weeks of supplementation, 6 ml of venous blood was collected for measuring serum 25-OH Vitamin D, serum calcium, serum phosphorous, serum malondialdehyde levels, and total antioxidant status. Vascular functions namely carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, arterial stiffness index, aortic pressures, and augmentation index were analyzed using periscope at baseline and after 8 weeks of Vitamin D supplementation in Vitamin D deficient type 2 diabetic patients in Department of Clinical Pharmacology, JIPMER using PeriScope (Genesis Medical Systems, India). The patients with Vitamin D insufficiency and normal Vitamin D levels were not given Vitamin D supplementation. However, their vascular functions were assessed at baseline and after 8 weeks of follow-up.

Statistical analysis

The sample size was calculated as 66 in Vitamin D deficient group for a power of 90%, alpha error of 5%, mean difference of 100 cm/s, standard deviation of 225 cm/s, and dropout rate of 20% using Power and Sample Size Calculation version 3.1.2. Copyright © 1997-2009 by William D. Dupont and Walton D. Plummer.^[16] Data were summarized as mean \pm standard deviation or median (interquartile range) for continuous variables. Categorical variables were summarized as percentages or ratios. Normality was assessed by Kolmogorov–Smirnov test. Change in serum 25-OH Vitamin D levels was compared by Wilcoxon signed rank test. Paired *t*-test and Wilcoxon signed rank test were used to compare the changes in vascular functions in each group depending on normal or nonnormal distribution, respectively. Changes in serum malondialdehyde and total antioxidant status were compared using paired *t*-test. Subgroup analysis was done to study the effect of Vitamin D supplementation in normotensive and hypertensive patients with type 2 diabetes mellitus and Vitamin D deficiency. *P* < 0.05 was considered statistically significant. Data were analyzed using IBM SPSS Statistics for Windows, Version 19.0, New York.

RESULTS

One hundred and three patients with type 2 diabetes mellitus were enrolled in the study from May 2015 to June 2016. Among them, 75 (72.82%) had Vitamin D deficiency, 23 (22.33%) had Vitamin D insufficiency, and 5 (4.85%) had normal Vitamin D levels [Figure 1]. The baseline characteristics of study participants are given in Table 1. Oral supplementation of 60,000 IU of cholecalciferol/week for 8 weeks in Vitamin D deficient group resulted in significant increase in serum 25-OH Vitamin D levels [Figure 2]. In addition, they had a significant decline in carotid-femoral pulse wave velocity, brachial-ankle pulse wave velocity, right brachial as well as right ankle arterial stiffness index, heart rate, and peripheral and central blood pressures [Table 2]. *Post hoc* subgroup analysis based on the presence or absence of hypertension showed similar findings in Vitamin D deficient patients with type 2 diabetes mellitus with

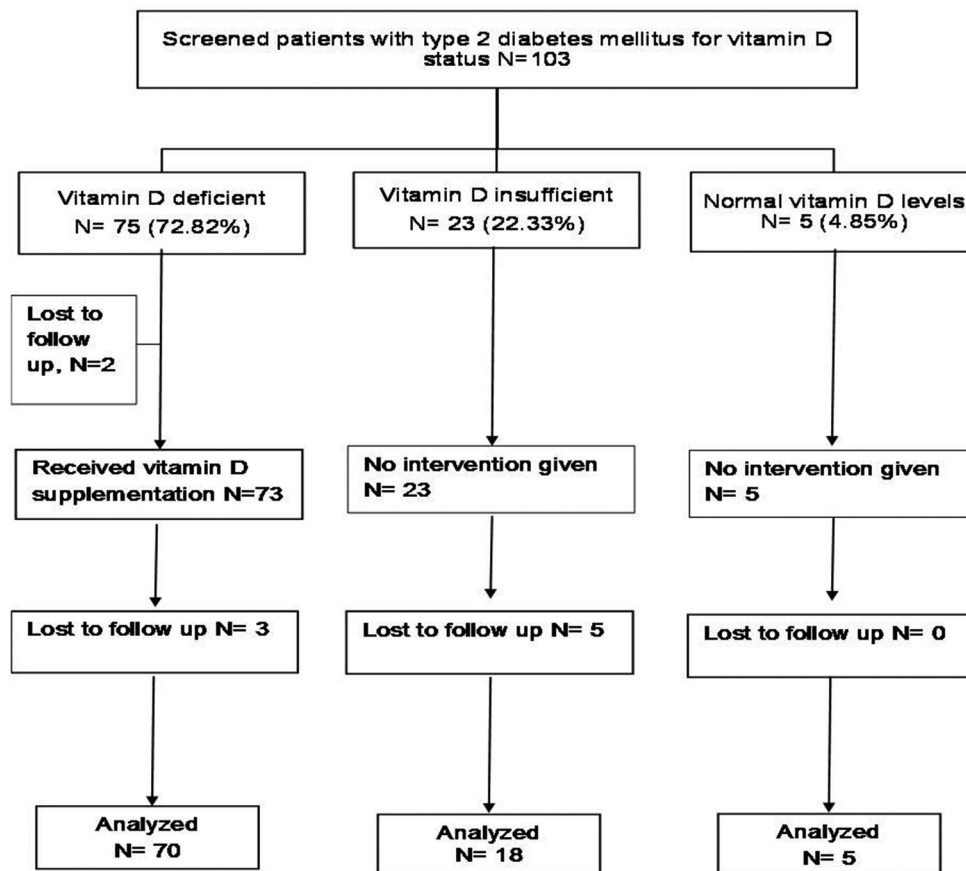


Figure 1: Patient flow diagram of the study

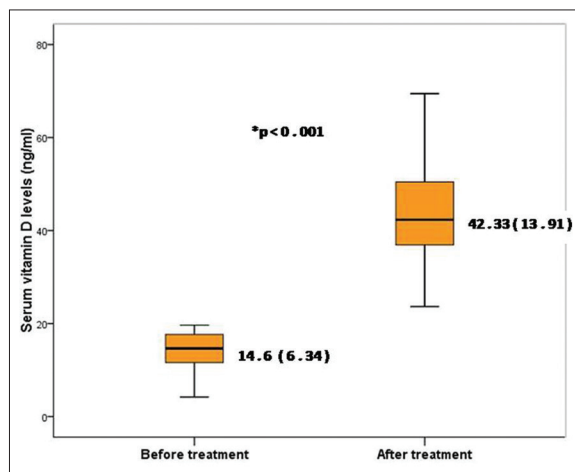


Figure 2: Change in serum Vitamin D levels after supplementation with 60,000 IU of cholecalciferol weekly for 8 weeks in patients with type 2 diabetes mellitus and Vitamin D deficiency ($n = 70$) *Wilcoxon signed rank test was used for statistical analysis. Values beside the boxes represent median (interquartile range). $P < 0.05$ was considered statistically significant

regard to their vascular functions [Tables 3 and 4]. Similarly, Vitamin D supplementation produced a significant fall in serum malondialdehyde levels and increase in total antioxidant status in the Vitamin D deficient group [Figures 3 and 4]. Likewise,

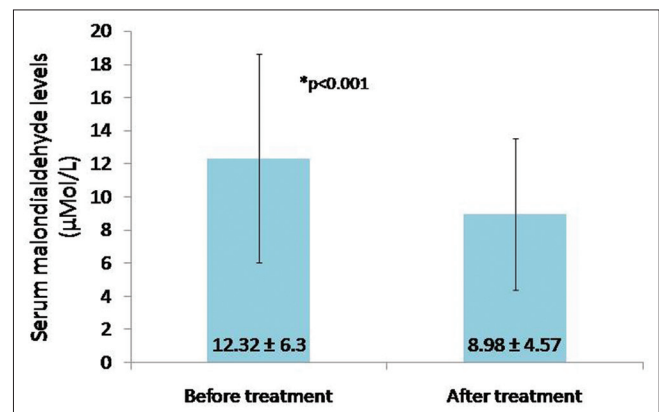


Figure 3: Change in serum malondialdehyde levels after supplementation with 60,000 IU of oral cholecalciferol weekly for 8 weeks in patients with type 2 diabetes mellitus and Vitamin D deficiency ($n = 70$) *paired t -test was used for statistical analysis. Values inside the bars represent mean \pm standard deviation. $P < 0.05$ was considered statistically significant

subgroup analysis of Vitamin D deficient patients based on the presence or absence of hypertension revealed significant reduction in malondialdehyde with increased total antioxidant status [Figures 5 and 6]. However, diabetic patients who were insufficient and normal for serum 25-OH Vitamin D levels did not show any significant difference in vascular function after 8 weeks of follow-up [Tables 5 and 6].

Table 1: Baseline characteristics of study participants (n=93)

Characteristics	Vitamin D deficient group (n=70)	Vitamin D insufficient group (n=18)	Normal Vitamin D group (n=5)
Age (years)	49.07±7.38	48.33±5.72	49.6±5.41
Serum 25-hydroxyvitamin D (ng/ml)	14.65 (6.34)	23.13 (5.23)	32.92 (2.85)
Male (%):Female (%)	37:63	78:22	60:40
Height (cm)	156.13±8	162.78±8.39	156.4±10.74
Weight (kg)	65.23±11.22	68±11.13	65.8±7.12
BMI (kg/m ²)	26.67±3.52	25.69±3.99	27.15±4.48
Hypertension (n [%])	35 (50)	11 (61.1)	2 (40)
Medications (n [%])			
Metformin monotherapy	14 (20)	4 (22.2)	1 (20)
Metformin and sulfonylurea combination	56 (80)	14 (77.8)	4 (80)
Antihypertensives	35 (50)	11 (61.1)	2 (40)
Atorvastatin	60 (85.7)	15 (83.3)	5 (100)
Daily exposure to sunlight (minutes)	60 (150)	330 (420)	480 (150)
Duration of type 2 diabetes mellitus (years)	4 (4.3)	5.5 (5.8)	4 (3.3)
Serum calcium (mg/dl)	9.15±0.56	9.45±0.65	9.94±0.87
Serum phosphorous (mg/dl)	3.48±0.45	3.24±0.27	4.08±2.4
Total cholesterol (mg/dl)	153.19±31.05	154.89±30.30	137.8±34.9
Triglycerides (mg/dl)	120.26±44.33	112.72±45.38	85.8±31.2
HDL cholesterol (mg/dl)	41.61±8.43	41.72±10.99	40.6±3.13
LDL cholesterol (mg/dl)	85.04±24.28	85.78±15.29	76.6±30
VLDL cholesterol (mg/dl)	23.8±8.51	22.44±9.16	20±6.4
Blood urea (mg/dl)	20.6±5.8	22.39±5.28	25.6±7.1
Serum creatinine (mg/dl)	0.9±0.1	0.9±0.1	1±0.1
Total bilirubin (mg/dl)	0.66±0.1	0.66±0.1	0.68±0.04
AST (IU/L)	20.76±5.15	19.39±5	22±3.9
ALT (IU/L)	25.73±9.11	23.39±10.33	26.4±3.78

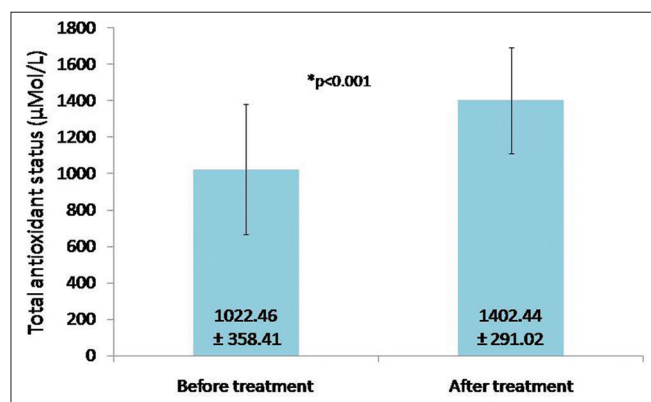


Figure 4: Change in total antioxidant status after supplementation with 60,000 IU of oral cholecalciferol weekly for 8 weeks in patients with type 2 diabetes mellitus and Vitamin D deficiency (n = 70) *paired *t*-test was used for statistical analysis. Values inside the bars represent mean ± standard deviation. *P* < 0.05 was considered statistically significant

Fatigue and sleepiness (n = 1) as well as loss of appetite for 2 weeks (n = 1) were the three adverse drug reactions that occurred with cholecalciferol supplements. However, the reactions subsided without any treatment and both the patients completed the study, and their results were used for analysis. During the study period, none of the participants reported any change in background medications.

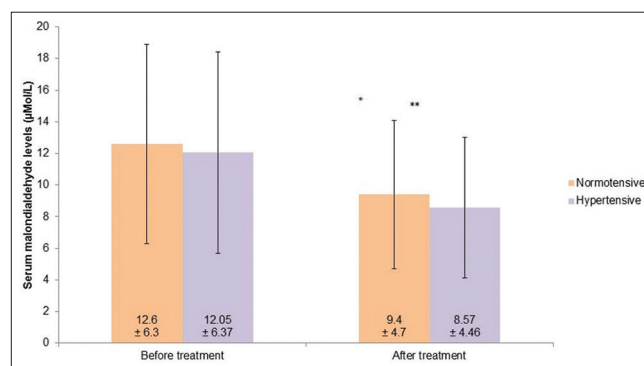


Figure 5: Change in serum malondialdehyde levels after supplementation with 60,000 IU of cholecalciferol/week for 8 weeks in normotensives (n = 35) and hypertensives (n = 35) with type 2 diabetes mellitus and Vitamin D deficiency. Paired *t*-test was used for statistical analysis; *P* < 0.05 was considered statistically significant. *In normotensives, comparing serum malondialdehyde levels before and after treatment, *P* = 0.001. **In hypertensives, comparing serum malondialdehyde levels before and after treatment, *P* < 0.001. Values inside bars represent mean ± standard deviation

DISCUSSION

This study has shown that Vitamin D supplementation reduces both central as well as peripheral blood pressure along with arterial stiffness as well as oxidative stress in diabetic patients with Vitamin D deficiency. Subgroup analysis performed in

Table 2: Effect of oral vitamin D₃ supplementation on vascular parameters in type 2 diabetic patients with vitamin D deficiency (n=70)

Parameter	Baseline	After 8 weeks	P
Heart rate (beats/min)	82.91±12.7	78.94±10.8	0.004
Systolic blood pressure (mm Hg)	130.34±13.9	123.55±14	<0.001
Diastolic blood pressure (mm Hg)	75.09±7.1	71.67±7.1	<0.001
Pulse pressure (mm Hg)	55.8±12.2	52.81±11.1	0.009
Carotid- femoral PWV (cm/s)	991.6±161.8	899.29±151.8	<0.001
Right brachial-ankle PWV (cm/s)	1446.16±204.3	1350.8±178.3	<0.001
Left brachial-ankle PWV (cm/s)	1493.8±219.6	1367.6±220.6	<0.001
Right brachial ASI [†] (mm Hg)	29.78 (11.37)	26.78 (9.2)	0.008
Left brachial ASI [†] (mm Hg)	28.4 (11.2)	28.28 (8.76)	0.401
Right Ankle ASI [†] (mm Hg)	39.68 (13.55)	38.28 (12.78)	0.023
Left Ankle ASI [†] (mm Hg)	41.45 (12.88)	40.78 (10.63)	0.346
Right Ankle Brachial Index	1.11±0.07	1.12±0.07	0.37
Left Ankle Brachial Index	1.1±0.07	1.1±0.07	0.86
Aortic systolic pressure (mmHg)	113.23±14.9	106.01±14.8	<0.001
Aortic pulse pressure [‡] (mmHg)	35.25 (14.25)	33 (12.13)	<0.001
Aortic diastolic pressure (mmHg)	75.26±7.4	71.45±7.2	<0.001
Aortic augmentation pressure (mmHg)	8.33±4.7	6.18±4.2	<0.001
Augmentation index [#] (%)	22.25 (10.13)	18.5 (13.13)	<0.001
Augmentation index @ Heart rate 75 [#] (%)	18.5 (12.88)	14.75 (14.13)	<0.001

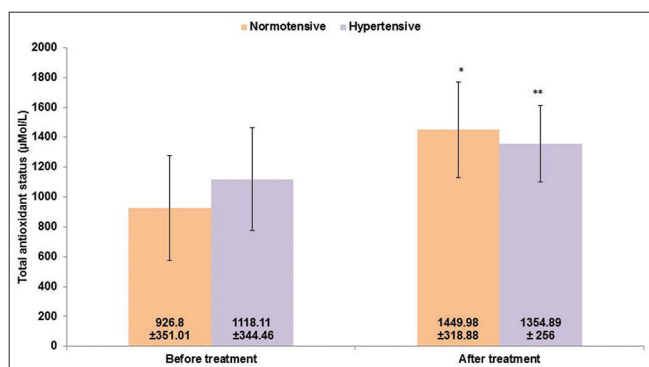


Figure 6: Change in total antioxidant status after supplementation with 60,000 IU of cholecalciferol weekly for 8 weeks in normotensive ($n = 35$) and hypertensive patients ($n = 35$) with type 2 diabetes mellitus and Vitamin D deficiency. Paired t -test was used for statistical analysis and $P < 0.05$ was considered statistically significant. *In normotensives, on comparing total antioxidant status before and after treatment, $P < 0.001$. **In hypertensive patients, on comparing total antioxidant status before and after treatment, $P = 0.002$. Values inside the bars represent mean \pm standard deviation

patients with type 2 diabetes mellitus and Vitamin D deficiency with or without hypertension to exclude the confounding effect of background antihypertensive medications on vascular functions also revealed similar reduction in blood pressure, pulse wave velocity, and serum malondialdehyde along with an increase in total antioxidant status.

There was a statistically significant reduction in carotid-femoral pulse wave velocity from 991.6 (161.82) cm/s at baseline to 899.29 (151.86) cm/s after 8 weeks of Vitamin D supplementation. Besides, there was a significant fall in the right and left brachial-ankle pulse wave velocity

from 1446.16 (204.33) cm/s to 1350.8 (178.39) cm/s and 1493.81 (219.65) cm/s to 1367.61 \pm 220.64 cm/s, respectively, after 8 weeks of Vitamin D supplementation. The findings of the present study were similar to a previous study that demonstrated significant decrease in pulse wave velocity from baseline (6.8 [1.55] m/s vs. 6.4 [1.3] m/s) following oral administration of 50,000 IU of Vitamin D₃/week for 8 weeks followed by 2000 IU/day for 4 weeks in patients with Vitamin D deficiency.^[17]

Our study found a significant reduction in central systolic, diastolic, and pulse pressure with Vitamin D supplementation. This is similar to another study that demonstrated significant decrease in systolic (115.8 [17.1]–106.3 [10.9]) and diastolic (75.4 [10.3]–68.5 [10.1] mmHg) blood pressures as well as pulse wave velocity (7.45 [1.55]–6.11 [1.89] m/s) in healthy participants following administration of 2000 IU/day of Vitamin D₃ supplementation for 14 days.^[18] A study in patients at risk for type 2 diabetes mellitus found significant decrease in pulse wave velocity by 73 cm/s (95% confidence interval of 142–3 cm/s) after oral supplementation of 100,000 IU of Vitamin D₃/month for 4 months compared to placebo.^[12] Similarly, a study by Dong *et al.* found that supplementation of 2000 IU Vitamin D₃/day for 16 weeks produced significant reduction in carotid-femoral pulse wave velocity from 5.41 \pm 0.73 m/s to 5.33 \pm 0.79 m/s ($P = 0.031$).^[19]

Various mechanisms have been suggested for the protective effects of Vitamin D on endothelial and vascular smooth muscle cells. Vitamin D is claimed to have anti-inflammatory action resulting in improved endothelial function and reduced arterial stiffness. It is proposed to have antiatherogenic effect owing to inhibition of foam cells formation. Moreover, regulation

Table 3: Effect of oral vitamin D₃ supplementation on vascular parameters in Vitamin D deficient patients with type 2 diabetes mellitus and hypertension (n=35)

Parameter	Baseline	After 8 weeks	P
Heart rate (beats/min)	81.98±13.74	78.13±10.8	0.04
Systolic blood pressure (mmHg)	132.33±15.37	125.09±16.08	0.003
Diastolic blood pressure (mmHg)	74.5±7.69	71.8±8.24	0.086
Pulse pressure (mmHg)	59.2±13.87	55.14±12.57	0.021
Carotid femoral PWV (cm/s)	1003.38±171.19	906.95±157.98	<0.001
Right brachial ankle PWV (cm/s)	1457.34±202.71	1337.37±172.35	<0.001
Left brachial ankle PWV (cm/s)	1510.88±235.58	1399.44±234.51	<0.001
Right brachial ASI [#] (mm Hg)	32.2 (14.3)	28 (8.75)	0.001
Left brachial ASI [#] (mm Hg)	31.5 (13.1)	29.25 (13)	0.566
Right ankle ASI (mm Hg)	45.76±10.81	41.7±12.4	0.034
Left ankle ASI (mm Hg)	46.37±11.47	44.25±12.23	0.253
Right ankle brachial index	1.12±0.07	1.13±0.07	0.147
Left ankle brachial index	1.1±0.07	1.11±0.07	0.623
Aortic systolic pressure (mm Hg)	115.94±17.25	108.7±17.71	0.003
Aortic pulse pressure (mm Hg)	40.16±11.15	36.09±10.37	0.002
Aortic diastolic pressure (mm Hg)	75.27±8.26	72.04±8.64	0.026
Aortic augmentation pressure (mm Hg)	9.41±5.24	6.94±4.7	<0.001
Augmentation index [#] (%)	23.5 (12.5)	19.5 (12.5)	<0.001
Augmentation index @ heart rate 75 [#] (%)	19 (14.5)	18.5 (16.5)	0.001

Table 4: Effect of oral vitamin D₃ supplementation on vascular parameters in normotensive patients with type 2 diabetes mellitus and Vitamin D deficiency (n=35)

Parameter	Baseline	After 8 weeks	P
Heart rate (beats/min)	83.83±11.88	79.75±11.01	0.042
Systolic blood pressure (mm Hg)	128.36±12.26	122.01±11.59	<0.001
Diastolic blood pressure (mm Hg)	75.67±6.64	71.54±6.01	<0.001
Pulse pressure (mm Hg)	52.4±9.27	50.47±9.2	0.189
Carotid femoral PWV (cm/s)	979.84±153.46	891.63±147.38	<0.001
Right brachial ankle PWV (cm/s)	1434.98±208.29	1364.22±185.76	0.009
Left brachial ankle PWV (cm/s)	1476.75±204.49	1335.79±204.25	<0.001
Right brachial ASI (mm Hg)	27.55±6.49	27.16±7.97	0.789
Left brachial ASI [#] (mm Hg)	26.8 (8.9)	26.65 (6.35)	0.507
Right ankle ASI [#] (mm Hg)	37 (8.35)	38.4 (8.75)	0.883
Left ankle ASI (mm Hg)	39.18±8.08	40.08±7.46	0.605
Right ankle brachial index	1.11±0.06	1.1±0.06	0.833
Left ankle brachial index	1.1±0.07	1.1±0.07	0.502
Aortic systolic pressure (mmHg)	110.51±11.84	103.31±11.01	<0.001
Aortic pulse pressure (mmHg)	34.71±7.6	31.91±7.56	0.022
Aortic diastolic pressure (mmHg)	75.24±6.58	70.86±5.7	<0.001
Aortic augmentation pressure (mmHg)	7.24±3.87	5.41±3.65	0.001
Augmentation index (%)	20.11±7.35	16.34±7.34	<0.001
Augmentation index @ heart rate 75 (%)	16.74±7.37	14.51±7.65	0.024

of blood pressure through modulation of renin–angiotensin system is postulated to be contributing to its beneficial effects on the vasculature.^[20]

Conversely, Gepner *et al.* showed that Vitamin D supplementation (2500 IU/day for 4 months) does not improve pulse wave velocity in postmenopausal women with serum Vitamin D levels ranging between 10 and 60 ng/ml.^[14] This could be attributed to wide variation in Vitamin D level of the

study population as well as inadequate dose or duration of treatment to confer cardiovascular benefits.

The present study showed a significant reduction in brachial-ankle pulse wave velocity following Vitamin D supplementation for a period of 8 weeks. This was in contrast to the findings of randomized controlled study done in patients with type 2 diabetes mellitus and suboptimal Vitamin D levels (<30 ng/ml).^[15] In that study, Vitamin D supplementation at a dose of 5000 IU/day for

Table 5: Vascular parameters in type 2 diabetic patients with Vitamin D insufficiency

Parameter	Baseline (n=18)	After 8 weeks (n=18)	P
Heart rate [#] (beats/min)	77.18 (16.06)	77.85 (10.06)	0.344
Systolic blood pressure (mm Hg)	125.03±11.1	125.14±12.7	0.963
Diastolic blood pressure (mm Hg)	75.03±8	73.67±8.03	0.375
Pulse pressure (mm Hg)	50±6.32	51.47±8.81	0.456
Carotid femoral PWV (cm/s)	1002.91±117.7	987.84±116.91	0.429
Right brachial ankle PWV (cm/s)	1458.78±153.92	1457.53±151.65	0.965
Left brachial ankle PWV (cm/s)	1508.37±158.62	1473.4±173.5	0.368
Right brachial ASI (mm Hg)	25.06±6.02	27.43±5.69	0.223
Left brachial ASI (mm Hg)	26.8±3.8	25.24±4.25	0.233
Right ankle ASI (mm Hg)	37.13±8.81	36.14±9.95	0.709
Left ankle ASI (mm Hg)	39.23±13.01	40.37±7.23	0.694
Right ankle brachial index	1.15±0.07	1.12±0.08	0.188
Left ankle brachial index	1.14±0.07	1.12±0.08	0.272
Aortic systolic pressure (mmHg)	108.56±10.17	107.81±11.02	0.71
Aortic pulse pressure [#] (mmHg)	32 (6.63)	35.25 (9.88)	0.796
Aortic diastolic pressure (mmHg)	74.5±7.23	73.5±7.38	0.39
Aortic augmentation pressure [#] (mmHg)	7 (3.75)	6.75 (5.5)	0.887
Augmentation index (%)	20.97±5.43	20.53±5.41	0.671
Augmentation index @ heart rate 75 (%)	20.03±6.57	20.08±7.76	0.965

Table 6: Vascular parameters in type 2 diabetic patients with normal Vitamin D levels

Parameter	Baseline (n=5)	After 8 weeks (n=5)	P
Heart rate (beats/min)	72.6 (18.53)	61 (10.38)	0.225
Systolic blood pressure (mm Hg)	124.5 (28.75)	126.5 (28.5)	0.225
Diastolic blood pressure (mm Hg)	66 (10.75)	68.5 (12)	0.042
Pulse pressure (mm Hg)	58.5 (18)	55 (20)	0.686
Carotid femoral PWV (cm/s)	994.25 (290.42)	1058 (281.63)	0.686
Right brachial ankle PWV (cm/s)	1524.9 (186.15)	1457.1 (383.85)	0.5
Left brachial ankle PWV (cm/s)	1425.5 (512.95)	1601.6 (400.23)	0.5
Right brachial ASI (mm Hg)	23.3 (18.83)	28.9 (14.37)	0.138
Left brachial ASI (mm Hg)	28.05 (10.48)	31.55 (7.97)	0.043
Right ankle ASI (mm Hg)	40.5 (11.85)	42.8 (14.45)	0.893
Left ankle ASI (mm Hg)	45.75 (9.77)	45.25 (11.78)	0.225
Right ankle brachial index	1.13 (0.15)	1.13 (0.14)	0.042
Left ankle brachial index	1.13 (0.11)	1.07 (0.14)	0.138
Aortic systolic pressure (mmHg)	106.5 (30.75)	106 (27.25)	0.345
Aortic pulse pressure (mmHg)	39 (16.5)	38 (17)	0.893
Aortic diastolic pressure (mmHg)	67 (13.5)	68 (14.25)	0.588
Aortic augmentation pressure (mm Hg)	8.5 (9.25)	9.5 (9)	0.5
Augmentation index (%)	23 (16.75)	24.5 (15.5)	0.786
Augmentation index @ heart rate 75 (%)	23.5 (12.25)	30 (17.75)	0.144

12 weeks did not alter brachial-ankle pulse wave velocity as well as flow-mediated dilatation which are markers of arterial stiffness and endothelial dysfunction, respectively. This could be due to the selection of patients with higher cutoff value of <30 ng/ml in contrast to the present study with a cutoff of <20 ng/ml of Vitamin D level. Similarly, a study evaluating effect of 2000 IU/day of Vitamin D₃ supplementation along with 200 mg/day of calcium for 24 weeks compared to only calcium (200 mg/day for 24 weeks) in patients with type 2 diabetes mellitus and Vitamin D deficiency found that Vitamin D supplementation did not produce any significant change in brachial-ankle pulse wave velocity.^[21] This

could be due to a lower total dose of Vitamin D supplementation used (336,000 IU) in their study.

In the present study, there was a significant reduction in augmentation index and augmentation pressure from baseline (22.25 [10.13] vs. 18.5 [13.13]% and 8.33 ± 4.7 vs. 6.18 ± 4.25 mmHg, $P < 0.001$) with Vitamin D supplementation. The results of this study were in consensus with findings of Zaleski *et al.* that showed significant reduction in augmentation pressure and augmentation index following oral Vitamin D supplementation of 4000 IU/day for 6 months.^[22] The reduction

in augmentation index and augmentation pressure was $12.3 \pm 5.3\%$ and 4 ± 1.5 mmHg, respectively. Similarly, Sünbül *et al.* demonstrated significant reduction in augmentation index from baseline (31 [14.5]% vs. 23 [22]%) with oral Vitamin D₃ supplementation (50,000 IU/week for 8 weeks and maintenance dose of 2000 IU/day for 4 weeks) in patients with Vitamin D deficiency.^[17] However, a randomized controlled study showed no change in augmentation index with Vitamin D supplementation (2500 IU/day for 4 months) in postmenopausal women with serum Vitamin D levels ranging between 10 and 60 ng/ml.^[14] Similarly, another randomized controlled trial evaluating the effect of Vitamin D supplementation (400 IU or 2500 IU/day for 6 months) showed no difference in aortic systolic blood pressure, aortic pulse pressure, and augmentation index between high dose and low dose Vitamin D group in healthy postmenopausal women.^[23]

Raised blood pressure is a major risk factor for the development of cardiovascular events such as myocardial infarction and stroke. It has been found that even a reduction of systolic blood pressure by 2 mmHg is clinically significant as it reduces mortality due to stroke and coronary artery disease by 6% and 4%, respectively.^[24] Our study has demonstrated statistically and clinically significant decrease in systolic and diastolic blood pressures from 130.34 (13.95) and 75.09 (7.16) mmHg at baseline to 123.55 (14) and 71.67 (7.16), respectively, with oral Vitamin D supplementation for 8 weeks. Our findings are similar to a study that evaluated the effect of oral 50,000 IU of Vitamin D₃ supplementation/week for 8 weeks on blood pressure in hypertensive patients with Vitamin D deficiency.^[25] In that study, patients in Vitamin D supplementation group demonstrated a significant reduction in systolic and diastolic blood pressures by 6.4 ± 5.3 and 2.4 ± 3.7 mmHg, respectively. The study also demonstrated a significant reduction in systolic and diastolic blood pressure from 121/80.5 mmHg at baseline to 110/76.3 mmHg after 12 weeks of Vitamin D supplementation in diabetic patients.^[26] Another study examining effect of Vitamin D supplementation in healthy population revealed a dose-dependent decrease in systolic blood pressure by 0.66 mmHg, 3.4 mmHg, and 4 mmHg following supplementation of Vitamin D₃ at doses of 1000, 2000, or 4000 IU/day for 3 months, respectively. However, the study did not find any change in diastolic blood pressure with Vitamin D supplementation.^[27]

Various mechanisms have been proposed for the antihypertensive effects of Vitamin D. It is found that Vitamin D modulates renin–angiotensin–aldosterone system and thus helps in regulation of blood pressure. It suppresses the expression of renin gene and thereby reduces production of angiotensin-II, a potent vasoconstrictor. In addition, Vitamin D is known to have renoprotective and anti-inflammatory effects which may also be contributing to its antihypertensive effects.^[20]

On contrary, in some studies, Vitamin D supplementation has not produced any alteration in blood pressure. A study

by Pilz *et al.* showed that oral Vitamin D supplementation of 2800 IU/day for 8 weeks in hypertensive patients with low Vitamin D levels (<30 ng/ml) did not produce any change in systolic blood pressure.^[28] The possible reason for this could be lower dose of Vitamin D supplementation inadequate to provide cardiovascular benefits. Moreover, the study evaluated the effect of Vitamin D supplementation in both Vitamin D deficient and insufficient patients.

Malondialdehyde, a marker of oxidative stress, and total antioxidant status, a measure of ability of biological sample like serum or plasma to tackle oxidative stress generated by the accumulation of free radicals, were used as markers of oxidative stress in our study. There was significant decrease in serum malondialdehyde levels and increase in total antioxidant status following Vitamin D supplementation in patients with type 2 diabetes mellitus and Vitamin D deficiency. This demonstrates the antioxidant action of Vitamin D, and this is in agreement with results of the randomized controlled trial by Foroozanfar *et al.*^[29] In that study, Vitamin D supplementation at doses of 50,000 IU/week for 8 weeks produced significant decrease in malondialdehyde level by 0.1 $\mu\text{mol/L}$ and increase in total antioxidant status by 22.5 mmol/L in Vitamin D deficient women with polycystic ovary syndrome.^[29] Another randomized controlled study in patients with nonalcoholic fatty liver disease showed that supplementation with 50,000 IU of Vitamin D₃ every 14 days for 4 months produced significant decrease in serum malondialdehyde by 2.09 ng/ml and an increase in total antioxidant status by 270 $\mu\text{mol/L}$.^[30] Similarly, another study revealed significant reduction in serum malondialdehyde from 4.7 to 2.9 ng/ml with Vitamin D₃ at a dose of 300,000 IU monthly for 3 months in Vitamin D deficient (<10 ng/ml) asymptomatic individuals.^[31] However, findings of Yiu *et al.* with Vitamin D supplementation of 5000 IU/day for 12 weeks in patients with type 2 diabetes mellitus did not improve oxidative stress markers.^[15]

The discrepancies in the results of various studies evaluating the effect of Vitamin D supplementation on vascular functions as well as oxidative stress could be attributed to differences in dose, route, duration of Vitamin D supplementation, techniques used to measure arterial stiffness, comorbidities in study participants, and use of concomitant drugs that modulate arterial stiffness.

The strengths of the study include paired study design to reduce interindividual variations and estimating serum 25-OH Vitamin D levels after 8 weeks supplementation as a measure of adherence. The limitation of the study is not including parathormone level, an important biomarker to assess response to Vitamin D.

CONCLUSION

The present study shows that oral Vitamin D supplementation of 60,000 IU/week for 8 weeks improves vascular functions and reduces oxidative stress in patients with type 2 diabetes mellitus and Vitamin D deficiency.

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Conflicts of interest

There are no conflicts of interest.

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