Effect of vitamin D replacement on cardiometabolic risk factors and electrophysiological pattern of peripheral neuropathy in vitamin D-insufficient or deficient type 2 diabetic patients

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Background
Diabetic neuropathy is one of the commonest chronic complications of diabetes seen in routine healthcare and considered the most common cause of peripheral neuropathy all over the world. Vitamin D (VD) deficiency is now recognized as a pandemic disease. This study was designed to explore the levels of 25-hydroxycholecalciferol [25(OH) D] in patients with type 2 diabetes mellitus (T2DM) with peripheral neuropathy. We also aimed to clarify the effect of VD supplementation on cardiometabolic status and electrophysiological pattern of peripheral neuropathy.

Patients and methods
This clinical trial enrolled 95 patients with T2DM with peripheral neuropathy. The enrolled patients were divided into three groups according to serum 25(OH) D levels. VD deficiency and insufficiency groups received VD supplements (42,000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks). Clinical, electrophysiological pattern, and laboratory parameters were evaluated at baseline and after 12 weeks of intervention. Serum 25(OH) D levels were measured by using a competitive enzyme-linked immunosorbent assay kit.

Results
Our results revealed that, among 95 patients with T2DM with peripheral neuropathy, 32 patients had VD insufficiency [20 ng/ml $<$25(OH) D $<$30 ng/ml], 50 patients had VD deficiency [25(OH) D $<$ 20 ng/ml], and 13 patients had VD sufficiency [25(OH) D $>$30 ng/ml]. Our results reported that 25(OH) D levels were negatively correlated with cardiometabolic risk factors and Toronto Clinical Scoring System. On the contrary, 25(OH) D levels were positively correlated with nerve conduction velocities (NCV). Stepwise multiple linear regression analysis revealed that glycated hemoglobin and Toronto Clinical Scoring System were the main predictors of 25(OH) D levels among other clinical and laboratory biomarkers. Logistic regression analysis observed that motor NCV and sensory NCV of median nerve and glycated hemoglobin were independent predictors of response to VD supplementation. NCV in studied groups showed that motor NCV and sensory NCV in the median, posterior tibial, and ulnar nerves were significantly decreased in both VD deficiency and insufficiency groups compared with VD sufficiency groups, and supplementation with 42 000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks improved cardiometabolic risk factors and electrophysiological pattern of peripheral neuropathy.

Conclusion
The supplementation of VD for 12 weeks to VD deficiency and insufficiency groups improved the cardiometabolic and electrophysiological pattern of peripheral neuropathy.

Keywords:
cardiometabolic, deficiency, electrophysiological, insufficiency, peripheral neuropathy, type 2 diabetes mellitus, vitamin D supplementation

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Introduction

A preponderance of evidence suggests that the most common cause of peripheral neuropathy in Egypt is diabetes mellitus (DM). Diabetic peripheral neuropathy (DPN) is one of the microvascular complications that cause morbidity and mortality in patients with DM; 50% of diabetic patients have diabetic neuropathy [1].

Several lines of evidence confirmed the DPN complications, of particular interest, foot amputation owing to painless foot ulcers, and diabetic autonomic

neuropathy may be associated with life-threatening conditions such as sudden death and silent myocardial ischemia [2]

Vitamin D (VD) is a fat-soluble vitamin that is naturally present in very few foods and is available as a dietary supplement. It is a steroid hormone with pleiotropic effects. In addition to the main effects of VD on bone and calcium metabolism, it has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation [3].

VD deficiency is now recognized as a pandemic disease. Its prevalence varies according to geographic location, season, ethnicity, and the standard laboratory value, of what is considered normal, deficient, and insufficient. VD deficiency is a risk factor for hypertension, diabetes, and various cancers [4]. Accumulating evidence suggests that VD deficiency contributes significantly to the pathogenesis of the two types of diabetes by impairing insulin secretion from pancreatic beta cells [5] and increasing insulin resistance [6]. Extensive data support a major role of VD deficiency in pathogenesis of diabetic neuropathies [7,8]. Therefore, the aim of the current study was to measure the levels of 25-hydroxycholecalciferol [25 (OH) D] in patients with type 2 diabetes mellitus (T2DM) with peripheral neuropathy. We also aimed to clarify the effect of VD supplementation on cardiometabolic status and electrophysiological pattern of peripheral neuropathy.

Patients and methods

Patients

This clinical trial was conducted in the outpatient clinics of diabetes and endocrinology of Internal Medicine Department at Zagazig University Hospital. This study included 95 patients with T2DM with peripheral neuropathy. The diagnosis of diabetes was according to ADA 2017; the enrolled diabetic patients were classified into three groups according to estimated serum 25(OH) D levels. VD deficiency was defined as serum 25(OH) D concentration of less than 20 ng/ml, insufficiency as 20 ng/ml less than 25(OH) D less than 30 ng/ml, and sufficiency was defined as 25(OH) D higher than 30 ng/ml [9]. The enrolled patients were subjected to full clinical assessment and neurological examination. There is no gold standard for the diagnosis of peripheral neuropathy. The expert panel of San Antonio conference recommends that it should be made on the basis of neuropathic symptoms, signs, and nerve conduction studies (NCS) [10].

Neurological examination was performed in the outpatient clinic of Neurology Department using the 10-g Semmes-Weinstein monofilament, applying the test on nine different sites on the plantar surface of the foot and diagnosing sensory neuropathy when less than seven sites were felt by the patient. Vibration perception threshold (VPT) was also measured, using a biothesiometer, to define the presence of diabetic neuropathy with a cutoff vibration perception threshold of more than 25 V for the diagnosis of loss of protective sensation.

Anthropometric variables including BMI were calculated as weight (kg)/height (m^2) and waist circumference (cm)/hip circumference (cm) ratio was measured. Body weight had to be stable for at least 3 months before study. All participants were on an unrestricted diet and were instructed not to modify their usual eating patterns.

Exclusion criteria included current psychiatric disorder that might affect the reliability of their response to the study questionnaire. The patients whose serum calcium was more than 10.4 mg/dl or those taking multivitamin supplementation or having hepatic, renal, or metabolic bone disorders (including parathyroid-related problems) were excluded from the study. Moreover, those patients with use of glucocorticoids or anti-seizure medications in the previous 6 months or those patients having history of malabsorption syndromes such as celiac disease or active malignancy or with active infection were excluded from the study. Patients with any neuropathic pain of nondiabetic origin including but not limited to lower back or neck pain (radiculopathy), postherpetic neuralgia, cancer-related pain, spinal-cord injury pain, multiple sclerosis pain, carpal tunnel syndrome pain, phantom pain, trigeminal neuralgia, or fibromyalgia were excluded from the study. In addition, we excluded pregnant patients.

The ethical committee of Faculty of Medicine, Zagazig University, approved our study protocol, and all participants signed a written informed consent before starting the study.

Severity of neuropathy

The severity of neuropathy was graded according to Toronto Clinical Scoring System (TCSS): 1–5 points for no neuropathy, 6–8 points for mild neuropathy, 9–11 points for moderate neuropathy, and 12–19 points for severe neuropathy. Symptom, reflex, and sensory tests, including pinprick, temperature, light

touch, vibration, and position sensation, were performed as part of the TCSS [11].

Nerve conduction study

NCS in the median, ulnar, peroneal, tibial, and sural nerves were carried out for all participants with the Micromed machine in the neurology outpatient clinic. Motor conduction studies were carried out with surface disk electrodes, with the active electrode inserted on the muscles and supramaximal stimulation of the corresponding nerves. Sensory NCS were performed. The parameters were compared between patients and controls. Individual values of conduction velocities, latencies, or amplitude were considered abnormal when outside the mean±2 SD of controls [12]. If two or more nerves had at least one abnormal parameter compared with the age-matched controls, it is electrophysiologically considered peripheral neuropathy.

Intervention

All VD insufficiency and deficiency patients received VD supplements 42 000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks to reach 25 (OH) D above 30 ng/ml and the maintenance dose of 1500–2000 IU per days.

Dietary intake

Dietary intakes of VD and calcium were ensured through 1-day 24-h dietary recall at the baseline and end of the eighth week of the study.

Blood sampling

Blood samples were drawn from all participants after an overnight fast and divided into three portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, for glycated hemoglobin (HbA1c), 1 ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2 : 1) for fasting plasma glucose (FPG), and serum was separated immediately from the remaining part of the sample and stored at −20°C until analysis.

Biochemical analysis

We determined FPG levels using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol, high-density lipoprotein-cholesterol, and triglycerides levels were measured by routine enzymatic methods (Spinreact). The low-density lipoprotein-cholesterol level was calculated using the Friedewald formula [13].

Determination of serum vitamin D levels

Serum concentrations of 25(OH) D were tested using enzyme-linked immunosorbent assay (Cat No. EQ. 6411–9601; Euroimmun Medizinische Labordiagnostika AG, Germany). Current recommendations define VD deficiency as serum 25 (OH) D levels less than 20 ng/ml and VD insufficiency less than 30 ng/ml [14].

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, Illinois, USA). Data were expressed using descriptive statistic (mean±SD) and were analyzed using one-way analysis of variance, followed by least significance difference test for multiple comparisons between groups. Pearson correlation coefficient was used to assess the association between serum 25(OH) D levels with anthropometric measures as well as electrophysiological parameters in the patients. A stepwise multiple linear regression analysis was done to detect the main predictors of serum 25(OH) D in T2DM group. Logistic regression analysis was performed to determine the predictor biomarker associated with VD supplementation among patients with T2DM. We considered P to be significant at less than 0.05, with a 95% confidence interval.

Results

Among 95 patients with T2DM with peripheral neuropathy, 32 patients had VD insufficiency [20 ng/ml <25(OH) D <30 ng/ml], 50 patients had VD deficiency [25(OH) D < 20 ng/ml], and 13 patients had VD sufficiency [25(OH) D >30 ng/ml].

Clinical, laboratory, and electrophysiological characteristics of the studied groups at baseline

VD insufficiency (n=32) and deficiency (n=50) patients had significantly long duration of diabetes and higher values of triglycerides, total cholesterol, low-density lipoprotein, FPG, HbA1c, PO_4, and TCSS compared with VD sufficiency (n=13) group. Furthermore, VD deficiency patients had significantly higher values of systolic blood pressures compared with VD sufficiency (P<0.001). On the contrary, VD insufficiency and deficiency patients had significantly lower values of high-density lipoprotein and serum calcium compared with VD sufficiency group (Table 1).

Electrophysiological tests of the studied groups

Nerve conduction velocities (NCV) in the studied group showed that motor nerve conduction velocities (MNCV) in median, ulnar, and posterior tibial (PT) nerves were significantly decreased in both VD insufficiency and deficiency patients compared with VD sufficiency group. Moreover, sensory nerve conduction velocities (SNCV) in median, ulnar, sural,

and PT nerves were significantly decreased in VD insufficiency and deficiency patients, with *P* value less than 0.001 (Table 1).

Regarding amplitudes, compound muscle action potential (CMAP) amplitudes in median, ulnar, and PT were significantly decreased in both obese patients with or without peripheral neuropathy compared with the control group. Sensory nerve conduction potential (SNAP) amplitudes in median, PT, and sural nerves were significantly decreased in decreased in VD insufficiency and deficiency patients compared with VD sufficiency group, with *P* value less than 0.001 (Table 1).

Correlations between serum 25-hydroxycholecalciferol levels with Toronto Clinical Scoring System as well as electrophysiological parameters in patients

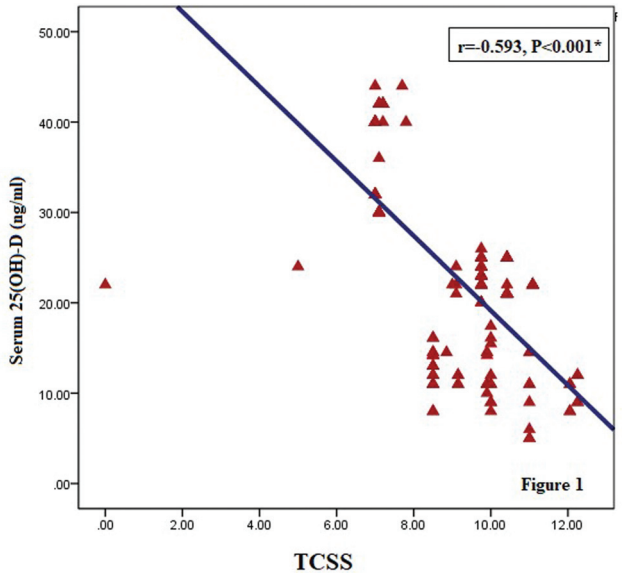
Our results reported that 25(OH) D levels were negatively correlated with cardiometabolic risk factors (Figs 1–4) and TCSS. On the contrary, 25(OH) D levels were positively correlated with electrophysiological tests:

Table 1 Clinical, laboratory, and electrophysiological characteristics of the studied groups at baseline

Variables	Vitamin D deficiency group (mean±SD) (*N*=50)	Vitamin D insufficiency group (mean±SD) (*N*=32)	Vitamin D sufficiency group (mean±SD) (*N*=13)	*P*1	*P*2
Age (years)	38.18±1.95	38.12±2.028	38.4±1.8	0.605	0.647
Duration of diabetes (years)	14.7±1.37	15.1±1.445	12.1±1.265	<0.001*	<0.001*
BMI (kg/m^2)	33.01±1.4	32.7±1.372	30.17±2.265	<0.001*	<0.001*
Waist/hip ratio	1.12±0.17	1.07±0.19	1.08±0.173	0.917	0.460
SBP (mmHg)	116.1±6.8	113.4±3.46	105.3±2.59	0.515	<0.05*
DBP (mmHg)	68.78±6.83	69.68±7.71	68.38±8.35	0.588	0.862
TC (mg/dl)	200.9±31.81	173.4±11.07	169.15±16.5	0.556	<0.001*
Triglycerides (mg/dl)	183.4±15.23	191.7±16.3	122.6±26.3	0.293	<0.001*
LDL-C (mg/dl)	197.6±24.5	176.6±7.8	167.2±19.7	0.179	<0.001*
HDL-C (mg/dl)	32.2±6.16	38.8±4.16	40.15±6.69	0.484	<0.001*
FPG (mg/dl)	164.4±20.4	143.4±14.84	141.4±11.9	0.751	<0.001*
HbA1c (%)	8.91±0.46	8.02±0.327	7.31±0.51	<0.001*	<0.001*
25(OH) D (ng/ml)	11.93±3.46	26.5±5.7	38.4±4.9	<0.001*	<0.001*
Ca (mg/dl)	7.71±0.46	8.14±0.32	8.51±0.5	0.015	<0.001*
PO_4 (mg/dl)	5.4±0.53	5.06±0.447	4.17±0.26	<0.001*	<0.001*
Alkaline phosphatase (IU/l)	99.47±13.1	96.06±10.89	102.6±16.6	0.079	0.371
TCSS	11.74±1.3	9.01±0.44	7.17±0.265	<0.001*	<0.001*
MNCV (m/s) (median)	41.32±11.9	46.37±10.02	48.19±10.54	<0.001*	<0.001*
PTN	46.6±6.32	58.7±11.27	54.09±10.3	<0.001*	<0.001*
CPN	46.14±12.77	49.93±16.8	55.09±10.54	0.027	0.807
Ulnar	43.0±8.91	50.7±15.59	54.4±10.64	<0.001*	<0.001*
SNCV (m/s) (median)	48.44±1.11	45.2±9.6	54.1±10.54	<0.001*	<0.001*
PTN	54.2±20.01	53.7±14.03	54.1±10.54	<0.001*	<0.001*
Ulnar	53.3±7.51833	54.2±14.24	55.7±11.67	<0.001*	<0.001*
Sural	43.6±15.6	49.8±14.1	53.2±10.54	<0.001*	<0.001*
CMAP amplitude (mV) (median)	2.14±0.11	3.2±1.8	6.4290±1.5	<0.001*	<0.001*
PTN	5.62±0.5	7.45±1.31	8.93±1.48	<0.001*	<0.001*
CPN	5.96±1.35	6.33±1.62	6.4±1.58	0.814	0.306
Ulnar	3.12±0.116	4.18±1.82	9.4±1.58	<0.001*	<0.001*
SNAP amplitude (μV) (median)	4.3±0.98	5.4±2.16	10.42±1.58	<0.001*	<0.001*
Sural	8.14±1.88	9.16±1.95	9.05±1.58	0.847	0.117
PTN	7.8±0.98	7.94±2.16	7.92±1.58	<0.001*	<0.001*
Ulnar	6.9±1.59	6.36±2.73	8.01±1.51	<0.001*	<0.001*

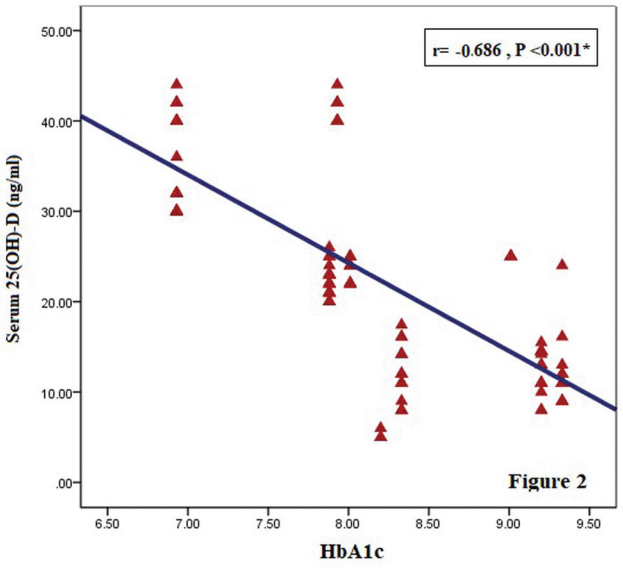
25(OH) D, 25-hydroxycholecalciferol; ALP, alkaline phosphatase; CMAP, compound muscle action potential; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FSI, fasting serum insulin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MNCV, motor nerve conduction velocity; PTN, posterior tibial nerve; SBP, systolic blood pressure; SNAP, sensory nerve conduction potential; SNCV, sensory nerve conduction velocity; TC, total cholesterol; TCSS, Toronto Clinical Scoring System. *P*1 compared vitamin D insufficiency with vitamin D sufficiency group. *P*2 compared vitamin D deficiency with vitamin D sufficiency group. *Statistically significant ($P<0.05$).

Figure 1



Correlations between serum 25(OH) D levels and TCSS. 25(OH) D, 25-hydroxycholecalciferol; TCSS, Toronto Clinical Scoring System.

Figure 2



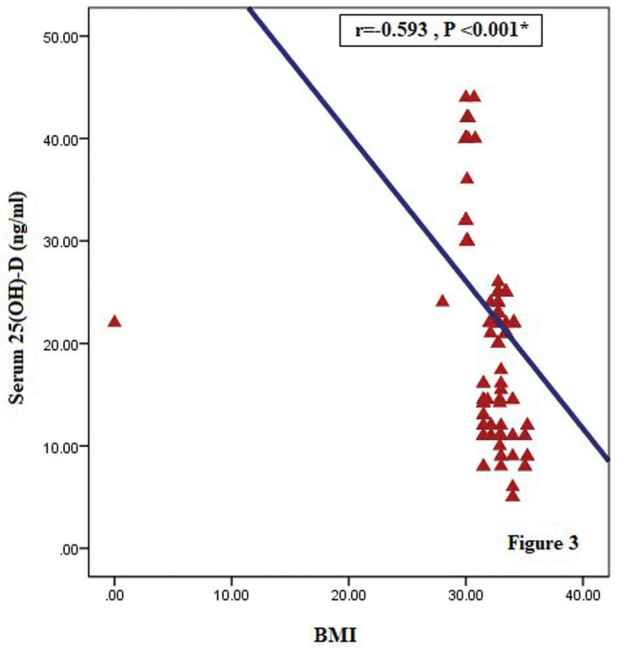
Correlations between serum 25(OH) D levels and HbA1c. 25(OH) D, 25-hydroxycholecalciferol; HbA1c, glycated hemoglobin.

MNCV (median, ulnar, and PT nerves), SNCV (median, ulnar, and PT nerves), CMAP amplitude (median, ulnar, and PT nerves), and SNAP amplitude (median, sural, ulnar, and PT nerve) (Table 2).

Linear regression analyses in patients with type 2 diabetes mellitus to assess the main independent parameters associated with serum 25-hydroxycholecalciferol levels

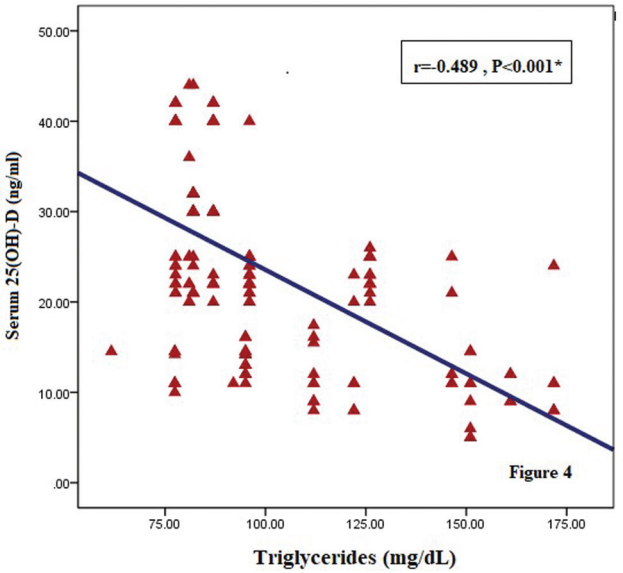
Linear regression analysis test revealed that serum 25 (OH) D levels were independently correlated with HbA1c and TCSS (P<0.001) (Table 3).

Figure 3



Correlations between serum 25(OH) D levels and BMI. 25(OH) D, 25-hydroxycholecalciferol.

Figure 4



Correlations between serum 25(OH) D levels and TG. 25(OH) D, 25-hydroxycholecalciferol; TG, triglycerides.

The effect of vitamin D supplementations on clinic-metabolic status and electrophysiological parameters of vitamin D deficiency and insufficiency groups

All VD insufficiency and deficiency patients received VD supplements 42 000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks to reach 25 (OH) D above 30 ng/ml.

In VD insufficiency group (n=32), after 12 weeks of trial, our results revealed significantly improvements of

TCSS and MNCV (median, ulnar, and PT nerves), SNCV (median, ulnar, and PT nerves), CMAP amplitude (median, ulnar, and PT nerves), and SNAP amplitude (median, sural, ulnar, and PT nerve), with *P* value less than 0.001 (Table 4).

Considering the effect of VD supplementation in VD deficiency group on cardiometabolic risks, our results revealed significantly improvement of glycemic and lipid profiles as well as calcium hemostasis. Moreover, TCSS and MNCV (median, ulnar, and PT nerves), SNCV (median, ulnar, and PT nerves), CMAP amplitude (median, ulnar, and PT nerves) and SNAP amplitude (median, sural, ulnar, and PT nerves) significantly improved after VD supplementations for 12 weeks, with *P* value less than 0.001 (Table 4).

Table 2 Pearson correlation between serum 25-hydroxycholecalciferol and electrophysiological parameters in patients with type 2 diabetes mellitus

	Serum 25(OH) D	
Electrophysiological parameters	*r*	*P*
MNCV		
Median	0.741	<0.001*
PTN	0.596	<0.001*
CPN	0.140	0.176
Ulnar	0.643	<0.001*
SNCV		
Median	0.741	<0.001*
PTN	0.319	<0.001*
Ulnar	0.646	<0.001*
Sural	0.066	0.527
CMAP amplitude		
Median	0.761	<0.001*
PTN	0.646	<0.001*
CPN	0.177	0.086
Ulnar	0.744	<0.001*
SNAP amplitude		
Median	0.703	<0.001*
PTN	0.703	<0.001*
Sural	0.271	<0.001*
Ulnar	0.617	<0.001*

25(OH) D, 25-hydroxycholecalciferol; CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; PTN, posterior tibial nerve; SNAP, sensory nerve conduction potential; SNCV, sensory nerve conduction velocity. *Statistically significant (P<0.05).

Assessment of the power of vitamin D supplementation to improve cardiometabolic risk and electrophysiological parameters

Logistic regression analysis was performed to detect the main predictors of cardiometabolic biomarkers associated with VD supplementation among patients with T2DM. Our findings revealed that among clinical and laboratory features, MNCV and SNCV of median nerve as well as HbA1c were independent predictors of improvement to VD supplementation, with odds ratios of 0.457, 2.123, and 520.042, respectively (Table 5).

Discussion

Despite attempts to increase awareness of the deleterious health consequences associated with T2DM and its complications, there continues to be a significant increase in the T2DM population worldwide. A growing body of evidence has corroborated that patients with T2DM are at high risk of microvascular complications including DPN, which has a bad effect on the quality of life, and it is associated with high mortality [15]. Substantial evidence assesses the prevalence of neuropathy in Egypt, and it found that the prevalence of neuropathy ranged from 21.9% in hospital outpatient clinics to 60% in hospital inpatients [16].

Past decades have witnessed a spurt in research activity that has shown a key role of VD in the pathogenesis of DPN. In fact, experimental studies demonstrated the associations between VD deficiency and low levels of nerve growth factors (neurotrophins), which are required for the development and survival of both sympathetic and sensory neurons and cause defective neuronal calcium homeostasis [14]. Decrease in

Table 3 Linear regression analyses in type 2 diabetes mellitus to test the influence of the main independent variables against serum 25-hydroxycholecalciferol (ng/ml) levels (dependent variable) in patients with type 2 diabetes mellitus

	Unstandardized coefficients		Standardized coefficients	*t*	*P* value	95% CI	
Model	*B*	SE	*β*			Lower bound	Upper bound
Constant	104.828	16.168		6.484	<0.001*	72.702	136.954
TC	−0.003	0.203	−0.007	−0.013	0.990	−0.406	0.401
TG	−0.046	0.184	−0.135	−0.249	0.804	−0.411	0.319
LDL	−0.008	0.047	−0.020	−0.160	0.873	−0.102	0.087
HbA1c	−6.691	0.961	−0.505	−6.961	<0.001*	−8.601	−4.781
TCSS	−2.414	0.535	−0.346	−4.516	<0.001*	−3.476	−1.352

CI, confidence interval; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein-cholesterol; TC, total cholesterol; TCSS, Toronto Clinical Scoring System; TG, triglycerides. *Statistically significant (*P*<0.05).

Table 4 Effect of vitamin D supplementation on clinical, laboratory, and electrophysiological characteristics of vitamin D deficiency and insufficiency groups

	Vitamin D deficiency group (mean±SD) (*N*=50)			Vitamin D insufficiency group (mean±SD) (*N*=32)		
Variable	Before	After	*P*	Before	After	*P*
BMI (kg/m^2)	33.01±1.4	31.3±1.62	0.01	32.7±1.372	30.7±5.81	0.007
Waist/hip ratio	1.12±0.17	1.06±0.12	0.320	1.07±0.19	1.08±0.19	0.887
SBP (mmHg)	116.1±6.8	115.9±5.867	0.835	113.4±3.46	113.5±3.1	0.906
DBP (mmHg)	68.78±6.83	71.5±7.25	0.060	69.68±7.71	70.4±7.2	0.669
TC (mg/dl)	200.9±31.81	183.3±23.3	<0.001*	173.4±11.07	173.3±10.1	0.941
TG (mg/dl)	183.4±15.23	100.6±26.3	<0.001*	191.7±16.3	90.6±15.07	0.850
LDL-C (mg/dl)	197.6±24.5	177.4±22.0	<<0.001*	176.6±7.8	169.3±18.2	0.736
HDL-C (mg/dl)	32.2±6.16	42.2± 3.1	<0.001*	38.8±4.16	40.01± 1.1	0.614
FPG (mg/dl)	164.4±20.4	146.4±20.4	<0.001*	143.4±14.84	142.3±12.5	0.768
HbA1c (%)	8.91±0.46	7.7±0.79	<0.001*	8.02±0.327	7.7±0.625	0.042
Ca (mg/dl)	7.71±0.46	8.2±0.51	<0.001*	8.14±0.32	8.3±0.44	0.141
PO_4 (mg/dl)	5.4±0.53	4.5±0.627	<0.001*	5.06±0.447	4.7±0.68	0.012
Alkaline phosphatase	99.47±13.1	95.03±8.73	<0.001*	96.06±10.89	94.8±9.2	0.670
TCSS	11.74±1.3	8.3±1.62	<0.001*	9.01±0.44	8.4±2.18	<0.001*
MNCV (m/s)						
Median	41.32±11.9	52.2±19.8	<0.001*	46.37±10.02	51.1±8.5	<0.001*
PTN	46.6±6.32	49.4±18.6	<0.001*	43.7±11.27	48.3±16.7	<0.001*
CPN	46.14±12.77	46.7±11.1	0.840	49.93±16.8	50.03±16.8	0.320
Ulnar	43.0±8.91	45.01±18.5	<0.001*	41.7±15.59	46.1±17.0	<0.001*
SNCV (m/s)						
Median	48.44±1.11	51.2±19.7	<0.001*	45.2±9.6	51.5±18.0	<0.001*
PTN	44.2±20.01	54.2±20.01	<0.001*	43.7±14.03	53.7±14.03	<0.001*
Ulnar	42.05±17.1	53.3±7.51	<0.001*	41.9±19.00	54.2±14.24	<0.001*
Sural	43.6±15.6	44.8±17.1	0.473	43.8±14.1	48±15.9	<0.001*
CMAP amplitude (mV)						
Median	5.62±0.5	5.9±3.42	<0.001*	3.2±1.8	5.9±3.1	<0.001*
PTN	5.96±1.35		<0.001*	7.45±1.31		<0.001*
CPN	6.12±0.116	6.3±1.52	0.188	6.33±1.62	6.4±1.56	0.662
Ulnar	4.3±0.98	6.8±3.42	<0.001*	3.18±1.82	6.9±3.18	<0.001*
SNAP amplitude (μV)						
Median	4.34±1.88	8.8±3.41	<0.001*	5.16±1.95	7.9±3.15	<0.001*
Sural	9.01±0.98	9.3±1.84	0.015	9.44±2.16	9.7±1.83	0.964
PTN	4.9±1.59	5.6±3.41	<0.001*	4.36±2.73	5.4±1.12	0.382
Ulnar	4.93±1.59	8.9±3.42	<0.001*	6.3±2.730	8.8±3.28	<0.001*

CMAP, compound muscle action potential; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MNCV, motor nerve conduction velocity; PTN, posterior tibial nerve; SNAP, sensory nerve conduction potential; SBP, systolic blood pressure; SNCV, sensory nerve conduction velocity; TC, total cholesterol; TCSS, Toronto Clinical Scoring System; TG, triglycerides. *Statistically significant (P<0.05).

Table 5 Logistic regression analysis evaluating the association of cardiometabolic risk and electrophysiological with vitamin D supplementation among patients with type 2 diabetes mellitus

	Unstandardized coefficients		Odds ratio	95% CI		*t*	*P* value
	B	SE		Lower bound	Upper bound		
Waist/hip ratio	–1.413	2.695	0.243	0.001	47.867	0.275	0.600
TC	0.068	0.061	1.070	0.949	1.206	1.219	0.270
HbA1c (%)	6.254	2.940	520.042	1.634	165490.8	4.524	0.033
MNCV of median	–0.783	0.339	0.457	0.235	0.888	5.346	0.021
SNCV of median	0.753	0.270	2.123	1.252	3.601	7.802	0.005
Constant	–0.695	2.508	0.499			0.077	0.782

CI, confidence interval; HbA1c, glycated hemoglobin; MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; TC, total cholesterol.

neurotrophins and defective calcium homeostasis increase nerve damage by toxins including hyperglycemia; in addition, VD receptor modulates neuronal cells differentiation and function [17]. In

this study, we aimed to explore the levels of 25(OH) D in patients with T2DM with peripheral neuropathy. We also aimed to clarify the effect of VD supplementation on cardiometabolic status and electrophysiological pattern of peripheral neuropathy.

The current study revealed that among 95 patients with T2DM with peripheral neuropathy, 32 patients had VD insufficiency [20 ng/ml <25(OH) D <30 ng/ml], 50 patients had VD deficiency [25(OH) D < 20 ng/ml], and 13 patients had VD sufficiency [25(OH) D >30 ng/ml].

In another Egyptian study conducted on Egyptian patients with or without DPN, the results revealed the mean serum levels of 25(OH) VD in patients with DPN were lower than that in patients without DPN. Moreover, we found that 87.6% of patients with DPN had VD deficiency [25(OH) D ≤28.3 ng/ml] compared with patients without DPN, whereas in their study, there were 45% who had VD deficiency [18].

The diverse results summarized before could be owing to different 25(OH) D cutoffs, as in our study, we classified our patients with T2DM with DPN as follows: VD deficiency was defined as serum 25(OH) D concentration of less than 20 ng/ml, insufficiency as 20 ng/ml less than 25(OH) D less than 30 ng/ml, and sufficiency was defined as 25(OH) D higher than 30 ng/ml.

Omics studies have indeed demonstrated that patients with DN had significant lower levels of VD than that in patient without DN, whereas there were similar values of VDBP and VDR in two groups of diabetic patients with and without DN [19].

Our results reported that 25(OH) D levels were negatively correlated with cardiometabolic risk factors and TCSS. On the contrary, 25(OH) D levels were positively correlated with NCV. Stepwise multiple linear regression analysis revealed that HbA1c and TCSS were the main predictors of 25(OH) D levels among other clinical and laboratory biomarkers. Logistic regression analysis observed that MNCV and SNCV of median nerve and HbA1c were independent predictors of response to VD supplementation.

Similar results were observed by Alamdari *et al*. [13]. They found that the serum 25(OH) D was significantly inversely correlated with the intensity of NCV impairment.

Against our results, Kheyami [20] demonstrated that VD has analgesic effect in patients with painful DPN, but VD deficiency does not more associate with painful neuropathy than painless neuropathy.

The results presented herein are innovative, as this study performs a robust estimation of NCV in patients with T2DM in relation to serum 25(OH) D. Even more importantly, our study evaluated the effect of VT supplementation on electrophysiological pattern. The results presented here are innovative, as we found that MNCV and SNCV in the median, PT, and ulnar nerves were significantly decreased in both VD deficiency and insufficiency groups compared with VD sufficiency groups, and after supplementation with 42 000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks, the cardiometabolic risk factors and electrophysiological pattern of peripheral neuropathy improved.Similar results were described in Putz *et al*. [21] who recommended VD supplementation in patients with diabetic neuropathy.

In earlier published studies conducted in our region, that is, Middle East, Zhang *et al*. [22] observed low VD in diabetes and the possible mechanisms for this association may include the presence of VD receptors in pancreatic beta cells to which circulating VD binds [23]. In addition, VD has been well recognized for its role in regulating extracellular calcium flux, and insulin secretion is known as a calcium-dependent process [24].

Conclusion

Our study observed that MNCV and SNCV in the median, PT, and ulnar nerves were significantly decreased in both VD deficiency and insufficiency groups compared with VD sufficiency group, and after supplementation with 42 000 IU oral VD per week and 500-mg calcium carbonate per day for 12 weeks, it improved cardiometabolic risk factors and electrophysiological pattern of peripheral neuropathy.

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Conflicts of interest

There are no conflicts of interest.

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