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Association between vitamin D status and malondialdehyde in T2DM patients with painful diabetic peripheral neuropathy

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ABSTRACT

The association of vitamin D with oxidative stress in type 2 diabetes mellitus (T2DM) patients with peripheral neuropathy (pDPN) has not been investigated in the literature yet. In this regard, we aimed to investigate the link between vitamin D status and malondialdehyde secretion in T2DM with pDPN. We included the T2DM patients with and without pDPN from a main tertiary medical diabetic center in Duhok City in this case-control investigation from September 2021 to March 2022. The patients aged between 40 and 70 years old. The patients were diagnosed based on the American Diabetes Association criteria. The T2DM patients with pDPN had a significantly lower level of vitamin D (12.10 ng/ml vs. 16.86 ng/ml; P=0.0013.) compared to the patients without compilations, respectively. The T2DM patients with pDPN had a significantly higher prevalence of severe deficiency (45.83% vs. 16.67%), while the patients without compilations had a significantly higher prevalence of deficient vitamin D (50.0% vs. 37.50%; P=0.0053). Moreover, the T2DM patients with pDPN had a significantly higher concentration of MDA compared to the T2DM patients without complications (30.55 nmol/ml vs. 16.6 nmol/ml; P=0.0098). The study did not find a significant correlation between MDA and vitamin D levels in T2DM patients with pDPN. This study showed that a higher concentration of MDA was not associated with lower vitamin D levels in T2DM patients with pDPN.

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Introduction

Diabetes mellitus is a metabolic condition that affects people around the world, and it can have serious health consequences. This disease is marked by high levels of sugar in the blood (hyperglycemia) that occur due to problems with insulin production, insulin resistance, or both. (1). Vascular complications, both microvascular (neuropathy, retinopathy, and nephropathy) and macrovascular (peripheral arterial disease, cardiovascular disease, and stroke), are the most significant effects of diabetes mellitus (2). The intricate process that results in cellular malfunctions in response to elevated glucose levels is not yet fully understood. However, it is known that neuropathy, which is damage to the peripheral nerves, affects about 10% of people who are diagnosed with type 2 diabetes mellitus (T2DM) in its early stages. Furthermore, up to 50% of individuals with long-term diabetes are affected by neuropathy. The likelihood of developing diabetic neuropathy is directly linked to the length of time and the seriousness of hyperglycemia (3).

Diabetic peripheral neuropathy (DPN) is the term used to describe the structural and functional impairments of the peripheral nervous system in diabetic patients, assuming that no other underlying causes are present. The pathogenesis of DPN is believed to be multifactorial, with several factors potentially contributing to its development, such as genetic susceptibility, endoneurial hypoxia or ischemia, heightened oxidative stress, increased glycosylation end-products, insufficient growth factors, and immune system dysfunction. However, the primary factor that initiates the development of DPN is long-term elevated blood glucose levels (4, 5). Moreover, a recent study found that vitamin D deficiency and higher glycated hemoglobin (HbA1c) were identified as predictive risk factors for painful DPN (6).

The development of diabetes-related late complications and pathogenesis is significantly impacted by oxidative stress. Free radicals are highly reactive molecules with one or more unpaired electrons, causing them to take electrons from other atoms or molecules to complete their outer energy levels. This reactivity makes free radicals highly unstable and a major contributor to oxidative stress (7). Malondialdehyde (MDA) is a key biomarker that is commonly used to assess the extent of lipid damage and oxidative stress resulting from free radical activity (8). Elevated levels of lipid peroxidation resulting from diabetes can lead to various long-term complications caused by peroxidative damage. These complications can include atherosclerosis and neural disorders (9, 10).

Vitamin D, a type of hormone that is soluble in fat, plays various physiological roles that extend beyond calcium metabolism (11). The global issue of vitamin D deficiency is prevalent among individuals who have prediabetes, type 2 diabetes mellitus, gestational diabetes, and obesity, which places them in a high-risk category (12).

The exact cause of diabetic peripheral neuropathy (DPN) is still unclear. However, recent research indicates

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that the lack of vitamin D, known as vitamin D deficiency (VDD), might be a contributing factor in the development of neuropathic complications. (13). However, the present association is a topic of controversy and has not obtained approval in other geographical regions (14). Therefore, it is worth noting that no previous studies in the literature have explored the possible connection between vitamin D levels and the secretion of malondialdehyde levels in Kurdish individuals with painful diabetic peripheral neuropathy in a real-world setting. Thus, the objective of this research was to investigate the link between vitamin D status and malondialdehyde secretion in Kurdish type 2 diabetic patients with painful diabetic peripheral neuropathy. We hypothesized that patients with painful diabetic peripheral neuropathy have lower vitamin D levels and a higher concentration of malondialdehyde (MDA) compared to non-complicated type 2 diabetes mellitus (T2DM) patients. Furthermore, we hypothesized that there is no significant correlation between MDA and vitamin D levels in T2DM patients with painful diabetic peripheral neuropathy (pDPN).

Materials and Methods

Study design and sampling techniques

The study was designed as a case-control investigation and patients were selected from a major tertiary medical center in Duhok City. The study period lasted from September 2021 to March 2022. Patients with T2DM with and without diabetic neuropathy were included in the study. Patients were eligible if they had been previously diagnosed with T2DM by an internist and had medical records available at the diabetic center. Patients were between 40 and 70 years old and were diagnosed based on the American Diabetes Association criteria. (15) . In this study, to obtain a representative sample of patients with T2DM in the Duhok diabetic center, we included patients from two clinicians. Furthermore, we attended for two days per week over an extended period. The choice of two days per week was made to ensure a representative sample and was based on technical considerations and feasibility. We assessed patients who visited the Duhok Diabetic Center for periodic medical check-ups or treatment to determine whether they met the eligibility criteria. In this study, we assessed 318 patients previously diagnosed with T2DM in the center at the Azadi Teaching Hospital in the Kurdistan region. Hence, we included 48 patients with painful DPN and 42 patients without painful DPN in this clinical case-control investigation after we considered all eligibility criteria. In addition, we matched the patients for age, gender, disease duration, and BMI as the criteria with possible effects on the outcomes. An internist (not included in this study) performed the clinical investigations with the aim of management of T2DM patients. The internist who did the clinical examinations has 15 years of experience in the management of patients with T2DM.

The setting of the study

We included the T2DM with and without painful DPN selected from the Duhok Diabetic Center in the Kurdistan Region. The center is the main clinical setting for the diagnostic, medical, and therapeutic services of patients with different endocrinology diseases in the Duhok Governorate. Other diabetic centers across the Duhok governorate are the primary medical care for diabetic patients only. The center is located inside the Azadi Teaching Hospital in Duhok City. Also, there is no private center for the management of endocrinology in this region. In this regard, It can be stated that the diabetic patients included in this study are a suitable and representative sample of diabetic patients in this particular region.

Inclusion and exclusion criteria

The initial eligibility criteria for this case-control study included T2DM patients of both genders, aged between 40 and 70, with and without painful DPN. In order to ensure successful matching, we employed narrow age intervals.

The patients who met any of the following criteria were excluded from the study: a positive diagnosis of COVID-19, other types of diabetes, chronic kidney disease, liver failure, malignancy, congestive heart failure, and general inflammation. Additionally, patients exhibiting symptoms of infection such as high fever, chills, and persistent coughing were also excluded. Patients with a history of allergies to medication or food, factors affecting nerves such as vasculitis, hypothyroidism, hyperparathyroidism, rheumatoid arthritis; recent intake of vitamin D or multi-vitamin supplements, causes of polyneuropathy other than diabetes, and central nervous system disorders were excluded. In addition, the patients with acute or chronic diabetic complications were excluded as well (diabetic ketoacidosis, retinopathy, nephropathy, and cardiomyopathy). We obtained the information from the clinical examinations, and medical records, and asked the patients.

Data collection and laboratory assessment

We collected detailed medical and clinical information from the patients before inclusion in the study. We obtained the following information from the patients; age, gender, disease duration, diabetes treatment, medications, medications received for DPN, smoking, dietary intake, and hypertension.

Biochemical assessment

An expert phlebotomist assisted the researchers in this study. He took the blood samples from the antecubital vein of the patients in the case of fasting overnight. The samples were centrifuged at 1,100 g for 15 minutes and were frozen at -30°C for the official analysis at the biochemistry laboratory. We obtained the blood samples over the cold seasons of this region (September 2021-March 2022). This assisted us in avoiding the seasonal fluctuation of vitamin D levels. The plasma level of malondialdehyde (MDA) was detected by using the MDA colorimetric assay kit (TBA Method). The MDA present in the plasma sample reacted with thiobarbituric acid (TBA) resulting in the formation of a colored compound (MDA-TBA complex) that was measured spectrophotometrically at 532 nm, (Cat.No.:E-BC-K025-S, Elabscience Biotechnology Co., Ltd, United States). We used the Cobas 6000 Roche Autoanalyzer (Roche Diagnostics, Munich, Germany) for the analysis of the blood samples. The first author of the study performed all biochemical tests at the Biochemistry Laboratory unit of Azadi Teaching Hospital in Duhok City in 2022. The following measures were recorded from the analysis; glycated hemoglobin (HbA1c), fasting blood sugar, total cholesterol, HDL-cholesterol, LDL-cholesterol, and trigyceride. Moreover, 25-hydroxyvitamin D [25(OH)

D] was measured from the collected blood samples.

The 25-(OH) D was analyzed based on the electrochemiluminescent immunoassay using Cobas 6000 Roche Autoanalyzer (Roche Diagnostics, Munich, Germany). Although there is no consensus on optimal levels of 25-hydroxyvitamin D, many researchers accept that 25-(OH)D ≥ 30 ng/mL (≥75 nmol/L) is considered sufficient; when 25(OH)D level is between 20–<30 ng/mL (50–<75nmol/L) is considered insufficient; deficiency of vitamin D is diagnosed when 25-hydroxyvitamin D is between 10–<20 ng/mL (25–<50 nmol/L); and if 25(OH)D<10 ng/mL is (<25 nmol/L), it demonstrates severe vitamin D deficiency. Vitamin D toxicity occurs when 25-hydroxyvitamin D levels are above 150 ng/mL (11, 16-19).

Assessment of diabetic peripheral neuropathy

We used the Michigan Neuropathy Screening Instrument for the assessment of DPN (MNSI). The scale is considered a DPN-approved screening tool with the following two unique purposes. It is a structured foot examination tool and has a 15-item self-administered questionnaire (MNSIE). We asked the patients to answer the questions one by one. In this regard, the first author asked the questions and recorded the responses in a pre-designed questionnaire. The scale evaluates abnormalities in appearance, ulcer presence, vibration, and ankle reflexes. In this study, MNSI and MNSIEs were performed by the first author after receiving sufficient training from the internist. We tried to avoid measurement bias by performing all assessments by the first author. The threshold for diagnosis of DPN is a score of ≥ 2 on the MNSIE out of a possible total score of 10 and a score of ≥ 7 on the MNSI for the presence of neuropathic symptoms. The readers can see the details of the assessment in the previous research (20).

Assessment of neuropathic pain

We used the DN4 scale with 10 items for the neuropathic pain assessment. The DN4 scale is a patient-reported symptom of sensory descriptors and signs connected with bedside examination. The French Neuropathic Pain Group established and validated the scale. The cut-off point of 4/10 had a specificity, sensitivity, and accuracy of 89.9% and 82.9%, 86.0%, respectively (21).

Anthropometric measures

We calculated the body mass index (BMI) based on the formula weight (kg)/height square (m^2). We used standard methods for the measurement of weight and height. We used the cut-off points of 102 cm and 88 cm for the recognition of obesity for male and female patients, respectively.

Ethical approval

The study adhered to the ethical guidelines established in the Declaration of Helsinki and was granted approval by the Local Ethics Committee of the Scientific Research Division, Directorate of Planning, Duhok Directorate General of Health in Iraqi Kurdistan (reference number 1062021 on June 1, 2021). All individuals who participated in the study provided their consent prior to being involved in the research project.

Statistical analyses

The study presented the general and medical characteristics of patients using the mean and standard deviation or number and percentage. To compare demographic, patient history, lifestyle, diet characteristics, and biochemical measurements between patients with type 2 diabetes mellitus with and without painful peripheral neuropathy, independent t-tests or Pearson chi-squared tests were conducted as appropriate. The study also examined the correlations between vitamin D and MDA, and between MDA and biochemical measurements among T2DM patients with neuropathy using the Spearman test. A p-value of less than 0.05 was considered significant. The statistical calculations were performed using JMP Pro 14.3.0. Statistical software (https://www.jmp.com/en_us/home.html).

Results

The study's results indicated that there were no significant differences in age (52.63 vs. 54.33 years; P=0.02562), gender (Male: 29.17% vs. 47.62%; P=0.0717), education (P=0.0948), and occupation (P=0.2579; Table 1) between

Table 1. Comparisons of demographic characteristics between type 2 diabetes mellitus with pDPN and without complications.

Characteristics	Study groups		p-value (two-sided)
	T2DM with pDPN (n=48)	T2DM without complications (n=42)	
Age Mean (SD)	52.63 (6.31) 40-65 years	54.33 (7.86) 40-70 years	0.2562[a]
Gender no (%)			
Male	14 (29.17)	20 (47.62)	0.0717[b]
Female	34 (70.83)	22 (52.38)	
Education no (%)			
Illiterate	31 (64.58)	17 (40.48)	
Under high school	12 (25.00)	17 940.48)	0.0948[b]
High school	4 (8.33)	4 (9.52)	
College Graduate	1 (2.08)	4 (9.52)	
Occupation no (%)			
Unemployed	1 (2.08)	1 (2.38)	
Business	2 (4.17)	0 (0.00)	
Housewife	32 (66.67)	21 (50.00)	0.2579[b]
Manual work	4 (8.33)	3 (7.14)	
Office work	5 (10.42)	9 (21.43)	
Retired	4 (8.33)	8 (19.05)	

[a] an independent t-test and [b] Pearson chi-squared test were performed for statistical analyses.
All patients were married.

T2DM patients with pDPN and those without complications. Additionally, T2DM patients with pDPN and those without complications exhibited similarities in terms of diabetic duration (P=0.09024), therapeutic aspects (P>0.05), and family history of the disease (Table 2). The study showed that the T2DM patients with pDPN had significantly higher levels of BMI (29.98 vs. 27.P=0.0078) compared to the patients without complications. But, the patients in both study groups were similar in the prevalence of overweight and obesity (P=0.1267). The study

Table 2. Comparisons of patient history between T2DM with pDPN and without complications.

Characteristics	Study groups		p-value (two-sided)
	T2DM with pDPN (n=48)	T2DM without complications (n=42)	
Diabetic duration			
1-5 yrs.	15 (31.25)	17 (40.48)	
6-10 yrs.	13 (27.08)	10 (23.81)	0.9024[b]
11-15 yrs.	13 (27.08)	9 (21.43)	
16-20 yrs.	4 (8.33)	4 (9.52)	
21-25 yrs.	3 (6.25)	2 (4.76)	
Diabetes treatment			
None	1 (2.08)	3 (7.14)	
Tablets	36 (75.00)	25 (59.52)	
Insulin	2 (4.17)	1 (2.38)	0.2246[b]
Diet	0 (0.00)	3 (7.14)	
Both diet and PA	0 (0.00)	1 (2.38)	
Both insulin and tab	9 (18.75)	9 (21.43)	
Daily dose of metformin (mg)			
≤ 1,000	16 (35.56)	13 (37.14)	0.8663[b]
1,000- < 2,000	25 (55.56)	20 (57.14)	
≥ 2,000	4 (8.89)	2 (5.71)	
Daily dose of insulin therapy (IU), long-acting			
≤ 10 IU	2 (50.00)	0 (0.00)	0.2231[b]
10- < 15 IU	1 (25.00)	2 (100)	
≥ 15 IU	1 (25.00)	0 (0.00)	
Daily dose of insulin therapy (IU), short-acting			
≤ 30 IU	1 (100)	1 (50.00)	1.0000[b]
30≤ 90 IU	0 (0.00)	1 (50.00)	
Daily dose of insulin therapy (IU), both fast-acting and long-acting			
≤ 30 IU	3 (42.86)	1 (33.33)	0.2699[b]
30< 90 IU	4 (57.14)	1 (33.33)	
≥ 90 IU	0 (0.00)	1 (33.33)	
Other medications			
Aspirin	5 (20.00)	4 (18.18)	
Dipeptidyl peptidase-4 inhibitor (Gliptins)	1 (4.00)	0 (0.00)	
Multi-medications	2 (8.00)	8 (36.36)	0.1301[b]
Statin	16 (64.00)	9 (40.91)	
Sulfonylurea	1 (4.00)	0 (0.00)	
Thiazolidinediones (Glitazones)	0 (0.00)	1 (4.55)	
Medications pattern			
Irregular	4 (8.51)	6 (16.67)	0.3176[b]
Regular	43 (91.49)	30 (83.33)	
Diabetic neuropathy treatment			
Carbamazepine (Tegretol)	5 (55.56)	0 (0.00)	N.A
Gabapentin (Neurontin)	2 (22.22)	0 (0.00)	
Pregabalin (Lyrica)	2 (22.22)	0 (0.00)	
Family history of T2DM			
Brother	5 (10.42)	2 (4.76)	
Father	0 (0.00)	1 (2.38)	
Mother	9 (18.75)	4 (9.52)	0.1476[b]
Multi-persons in family	20 (41.67)	16 (38.10)	
None	11 (22.92)	9 (21.43)	
Sister	3 (6.25)	6 (14.29)	
Wife/Husband	0 (0.00)	4 (9.52)	
Metformin therapy (years)	8.84 (6.25)	9.23 (6.13)	0.7823[a]
Metformin with insulin therapy (years)	5.57 (1.62)	10.38 (6.07)	0.0641[a]

[a] an independent t-test and [b] Pearson chi-squared test was performed for statistical analyses.

showed that the T2DM patients with pDPN had significantly higher concentrations of FBS (238.47 vs. 172.22; P<0.0001), cholesterol (204.72 vs. 170.83; P=0.003), LDL (130.95 vs. 108.08; P=0.0069), T.G. (216.32 vs. 146.07; P<0.0001), and HBA1c (10.38 vs. 8.35; P<0.0001). But, the T2DM patients with pDPN and without complications were similar in SBP (P=003524), DBP (P=0.080), WC (P=0.1996), and HDL (P=0.4199; Table 3).

The T2DM patients with pDPN had a significantly lower level of vitamin D (12.10 ng/ml vs. 16.86 ng/ml; P=0.0013.) compared to the T2DM patients without compilations, respectively. The T2DM patients with pDPN had a significantly higher prevalence of severe Vitamin D deficiency (45.83% vs. 16.67%), while the patients without compilations had a significantly higher prevalence of deficient vitamin D (50.0% vs. 37.50%; P=0.0053). Furthermore, the T2DM patients with pDPN had a significantly higher concentration of MDA compared to the patients without complications (30.55 nmol/ml vs. 16.6 nmol/ml; P=0.0098; see Table 4 and Fig. 1). The study did not find a significant correlation between MDA, vitamin D levels and other biochemical measurements in T2DM patients with pDPN, as shown in Table 5 and Figure 2.

Discussion

This study showed that the T2DM patients with pDPN had a significantly lower concentration of vitamin D, higher rates of severely deficient vitamin D, and higher concentration of MDA compared to the T2DM patients without complications. The concentration of MDA was not related to vitamin D in T2DM patients with pDPN.

Polyneuropathy is widely regarded as one of the most prevalent and serious microvascular complications oxidative stress is known to be a contributing factor in the

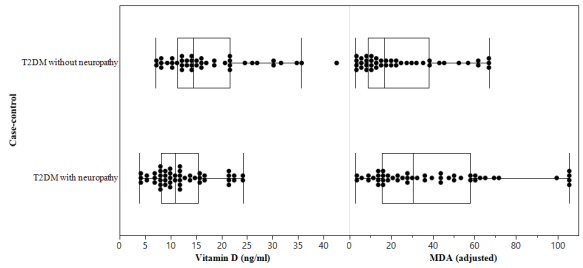

Figure 1. Vitamin D and MDA levels of study groups.

Table 3. Comparisons of biomedical measurements between T2DM with pDPN and without complications.

Characteristics	Study groups		p-value (two-sided)
	T2DM with pDPN (n=48)	T2DM without complications (n=42)	
SBP (mmHg) (SD)	13.20 (1.36)	12.93 (1.31)	0.3524[a]
DBP (mmHg) (SD)	8.63 (0.61)	8.39 (0.64)	0.0880[a]
BMI (kg/m^2) (SD)	29.98 (5.05)	27.30 (3.90)	0.0078[a]
BMI category no (%)			
Normal weight	6 (12.50)	12 (28.57)	0.1267[b]
Overweight	23 (47.92)	19 (45.24)	
Obese	19 (39.58)	11 (26.19)	
WC (cm) (SD)	106.37 (9.48)	103.41 (11.81)	0.1996[a]
FBS (mg/ml) (SD)	238.47 (67.58)	172.22 (55.39)	<0.0001[a]
Total cholesterol (mg/ml) (SD)	204.72 (49.33)	170.83 (33.19)	0.0003[a]
HDL-C (mg/ml) (SD)	45.94 (8.73)	44.36 (9.39)	0.4199[a]
LDL-C (mg/ml) (SD)	130.95 (43.28)	108.08 (33.36)	0.0069[a]
T.G (mg/ml) (SD)	216.32 (86.83)	146.07 (62.60)	<0.0001[a]
HbA1c % (SD)	10.38 (1.89)	8.35 (1.62)	<0.0001[a]

[a] an independent t-test and [b] Pearson chi-squared test was performed for statistical analyses.
The red numbers show significant differences.

Table 4. Comparisons of vitamin D measurements and MDA levels between T2DM with pDPN and without complications.

Characteristics	Study groups				p-value (two-sided)
	T2DM with pDPN (n=48)		T2DM without complications (n=42)		
	Mean (SD)	95% CI	Mean (SD)	95% CI	
Vitamin D (ng/ml)	12.10 (5.60)	10.47 to 13.73	16.86 (7.84)	14.39 to 19.33	0.0013[a]
Vitamin D category no (%)					
Severe deficient	22 (45.83)	32.58 to 59.71	7 (16.67)	8.32 to 30.60	0.0053[b]
Deficient	18 (37.50)	25.22 to 51.64	21(50.00)	35.53 to 64.47	
Insufficient	8 (16.67)	8.70 to 29.58	9 (21.43)	11.71 to 35.94	
Sufficient	0 (0.00)	NA	5 (11.90)	5.19 to 25.00	
MDA(nmol/ml) median (IQR)	30.55 (42.60)	30.66 to 47.76	16.6 (29.29)	18.20 to 30.88	0.0098[a]

[a] an independent t-test and [b] Pearson chi-squared test was performed for statistical analyses.
The red numbers show significant differences.

Table 5. Correlations of MDA levels with biochemical measurements among T2DM patients with pDPN.

Variable	by Variable	Spearman ρ	p-value	Presentations
SBP (mmHg)	MDA	0.0069	0.9632	
DBP (mmHg)	MDA	-0.0957	0.5223	
BMI (kg/m^2)	MDA	-0.1699	0.2484	
WC (cm)	MDA	-0.2814	0.0526	
FBS (mg/ml)	MDA	0.0980	0.5075	
Total cholesterol	MDA	0.1701	0.2478	
HDL-C (mg/ml)	MDA	0.1418	0.3363	
LDL-C (mg/ml)	MDA	0.1200	0.4166	
T.G (mg/ml)	MDA	-0.1178	0.4252	
Vitamin D (ng/ml)	MDA	0.1831	0.2129	
HbA1c (%)	MDA	-0.0366	0.8048	

Nonparametric Spearman's ρ test was performed for statistical analyses.

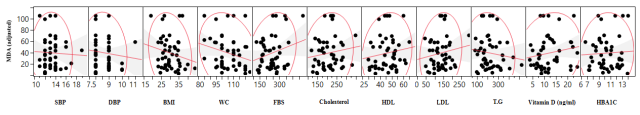

Figure 2. Scatter plot of correlations of MDA levels with biochemical measurements among T2DM patients with pDPN.

development of polyneuropathy in individuals with diabetes. The occurrence of oxidative stress is a result of an imbalance between reactive oxygen species (ROS) and antioxidants, leading to increased radical production and decreased antioxidant rates (22, 23). MDA plays a role in lipid peroxidation (24) and is commonly utilized as a biomarker in the assessment of oxidative stress among T2DM patients (25, 26). In chronic illnesses like T2DM and its complications, an abundance of MDA is typically produced (27). It has been reported that there is a lower concentration of MDA in T2DM patients with painful diabetic neuropathy (PDN) compared to both the T2DM group and the control group. However, the reason for the reduced MDA concentration in patients with diabetes and neuropathy is not yet clear (28). Daily use of alpha lipoic acid, vitamin A, C, and E supplements could be related (29). The use of vitamin supplements such as vitamins A, C, and E on a daily basis is considered to be one of the strategies for managing or controlling T2DM in patients with diabetic DPN. While there is limited information available on the role of vitamin A and vitamin C supplements in this context, studies have demonstrated the potential benefits of vitamin E for diabetic patients with peripheral neuropathy. These benefits include a reduction in the final products of lipid peroxidation, such as MDA (30, 31). In addition to vitamin supplements, the study investigated the impact of simvastatin medication on patients with T2DM and DPN and observed a significant decrease in the MDA biomarker levels (32).

According to existing literature, there is a substantial increase in the MDA level among individuals with T2DM and DPN when compared to the control group (33). However, MDA levels were significantly lower in the T2DM patients with DPN compared to T2DM and healthy control groups (28). In this particular study, patients who received supplements such as vitamins A, C, E, and D supplements, or who took alpha-lipoic acid supplements, were excluded from the study. However, it should be noted that other studies in the literature may not have excluded patients who were taking supplements. This difference in study design could potentially affect the results and should be taken into consideration when interpreting and comparing findings between studies (28). The literature has confirmed that vitamin D deficiency and insufficiency are related to painful diabetic neuropathy either in studies (34, 35) and meta-analyses (36).

Prior research has indicated that diabetic neuropathy is linked to reduced expression of nerve growth factor (NGF) in human diabetic nerves. Furthermore, it has been demonstrated that the introduction of exogenous NGF can partially reverse some of the pathological changes that occur in diabetic nerves (37, 38). At the same time, it is known that vitamin D can stimulate the production of nerve growth factor (NGF) in human cell lines (39).

Thus, vitamin D may have a protective effect against neuropathy in diabetic patients by promoting NGF synthesis. An experiment conducted on streptozotocin-diabetic rats showed that a derivative of vitamin D3 induced NGF synthesis and prevented deficits in neurotrophic factors. This suggests that vitamin D supplementation may have a beneficial effect in preventing or treating neuropathy in individuals with diabetes (40). Also, vitamin D supplement has been shown to lead to significant improvement in neuropathic pain management in diabetic patients (41) as well as a reduction in neuropathy symptom scores (42). Several clinical trials have shown that vitamin D supplementation can improve both pain and neuropathy-specific quality of life in diabetic patients. Furthermore, another study has also concluded that vitamin D is an independent risk factor for the presence and severity of diabetic neuropathy (43). Whether there is an association between vitamin D and oxidative or not, we need to consider the physiology of vitamin D.

Strengths and limitations of the study

Certain aspects have been reinforced in the current clinical case-control study. We aimed to enhance homogeneity across several factors, such as age, gender, disease duration, treatments, doses, and family history of T2DM, to the greatest extent possible. Furthermore, we took steps to minimize the influence of factors associated with the outcomes under investigation. For instance, we excluded patients displaying signs of infection or those with a history of allergies to medication or food. Additionally, we

ensured the inclusion of patients spanning a wide range of socio-demographic characteristics over an extended timeframe. However, the study was not exempt from the limitations. We did not measure other oxidative stress in this study. Other oxidative stress factors could be related to vitamin D concentrations in T2DM patients with pDPN.

This study showed that the T2DM with pDPN had a significantly lower concentration of vitamin D and a higher concentration of MDA compared to T2DM without complications. The higher concentration of MDA was not associated with vitamin D in T2DM with pDPN.

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Conflicts of interest
The authors declare no conflict of interest.

Contributions
Alan Bapeer Hassan: Concept, review, design, data collection, assessment, first draft, analysis

Ali Hussein Ahmad Al-Dosky
Review, supervision, methods, critical review, analysis.

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