




RESEARCH ARTICLE

REVISED The impact of vitamin D supplementation on peripheral neuropathy in a sample of Egyptian prediabetic individuals

[version 2; peer review: 2 approved]

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Abstract

Background: Vitamin D deficiency is seen more frequently in diabetic patients with distal symmetrical polyneuropathy. Unfortunately, there is a shortage of data concerning prediabetic individuals with peripheral neuropathy (PN). Therefore, we aimed to study the association of vitamin D deficiency with PN severity and to determine the effect of vitamin D supplementation on PN in prediabetics.



Methods: A case-control study was conducted consisting of 89 prediabetic individuals with PN and a control group of prediabetics without PN, recruited from the outpatient department of the National Institute of Diabetes and Endocrinology, Cairo, Egypt. All patients were screened for PN using clinical examination and Douleur Neuropathique 4 diagnostic questionnaire (DN4). Group A (with PN) was assessed for neuropathic severity using the Short-Form McGill Pain Questionnaire (SF-MPQ). In addition, 25-hydroxyvitamin D, ionized calcium, phosphorus, parathyroid hormone (PTH), glycated hemoglobin (HbA1c), fasting blood glucose (FBG), 2-hour post 75g glucose (2h-PPBG) and lipid profile were measured for both groups. Prediabetic patients with PN were given vitamin D3 200,000 IU IM monthly for three months. After three months, clinical assessment, DN4, SF-MPQ and all laboratory measures were repeated.

Results: Vitamin D was not associated with the severity of PN patients. However, supplementation of vitamin D resulted in a highly significant improvement in glycemic parameters, $p \leq 0.001$. Interestingly, neuropathy score and severity before vitamin D supplementation were (6.4 ± 1.6 and 28.3 ± 7.2) and after became (2.5 ± 0.9 and 17 ± 6.3 , $p \leq 0.001$).

Conclusion: Correction of vitamin D deficiency in prediabetics with PN as well as hypovitaminosis D, improves glycemic parameters, PN score and severity.

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Keywords

prediabetes, vitamin D, peripheral neuropathy, neuropathic score

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REVISED Amendments from Version 1

1. Further clarification of the study design: An analytic case-control followed by an interventional one arm clinical trial design (quasi experiment) were performed to fulfill the preset objectives. The case-control study included 89 prediabetic patients with peripheral neuropathy (Group A) and 89 prediabetics without peripheral neuropathy (Group B). Patients of group A were invited to participate in a quasi-experiment by administration of therapeutic dose of vitamin D. None of the 89 patients participating in the case-control study refused to be enrolled in the interventional study
2. Laboratory studies included: ionized calcium, phosphorous, PTH, and 25(OH)vitamin D, FBG, 2h-PPBG, glycated hemoglobin and total lipid profile (total cholesterol, low density lipoprotein (LDL) and triglycerides)
3. During the study, we found few patients with ionized calcium 0.8 mmol/L. they were 11 out of 178 prediabetic patients. They were asymptomatic (mostly due to chronic hypocalcemia) and once they were recognized to be hypocalcemic, they were given the proper management.
4. **Table 1** is updated (removed TSH/ creatinine and FBG added)
5. **Table 2** is updated (p=0.095 NS)
6. Vitamin D level showed a negative but statistically insignificant correlation with the severity of peripheral neuropathy (assessed by SF-MPQ) ($r = 0.032$, $p = 0.766$) and pain score (assessed by DN4 questionnaire) ($r = 0.052$, $p = 0.629$). Figure 1a/1b removed and discussion and conclusion are updated to new findings.
7. **Table 4** changed (MNB=McNemar Bowker test between patients with peripheral neuropathy before and after vitamin D injection. Paired t-test in both Short McGill and DN4 <0.001)
8. Underlying data DOI updated
9. References have been modified accordingly

Any further responses from the reviewers can be found at the end of the article

Editorial note:

After being made aware of issues with this article, the F1000Research Editorial Team worked closely with the authors' institution, who completed a rigorous investigation of the concerns raised. Investigation was carried out by a multidisciplinary internal auditing committee formed of endocrinology and public health individuals across the Faculty of Medicine - Ain Shams University. The committee reviewed the study datasets and analyses and their findings identified various inaccuracies that require correction. In line with our [article policies](#) around permanency of content, in order to maintain the integrity and completeness of the scholarly record, F1000Research allows authors to publish revised versions, and any errors that become apparent during peer review or later can be corrected through the publication of new versions. We have worked with the article authors towards correction of the manuscript and publication of a new version. Corrections and changes relative to the previous version are clearly summarized in the 'Amendments' section at the start of the new version.

Introduction

Diabetes mellitus (DM), a significant world health problem, is a metabolic disease, which occurs due to a defect in

insulin release and or insulin resistance¹. Globally, the prevalence of type 2 diabetes (T2DM) is high and rising across all regions².

There is a higher frequency of idiopathic polyneuropathy, small fiber neuropathy and painful sensory neuropathy among prediabetics. These findings suggest an involvement of the small unmyelinated nerve fibers that carry pain, temperature, and regulate autonomic function during prediabetes, before the development of diabetes³.

Vitamin D, which is a fat-soluble hormone, has multiple physiological roles, which extends far beyond calcium metabolism⁴. Vitamin D deficiency is a worldwide health problem, patients with prediabetes, T2DM, gestational diabetes and obesity represent a high-risk group⁵.

Recently, a lot of studies have been done to assess the association between vitamin D level and the diabetic peripheral neuropathy in patients with diabetes mellitus and to study the effect of vitamin D on painful neuropathy, but there is a lack of data concerning prediabetic individuals¹.

The aim of this work was to determine the association of vitamin D deficiency with peripheral neuropathy severity and evaluate the effect of vitamin D supplementation on peripheral neuropathy in prediabetics with hypovitaminosis D.

Methods

An analytic case-control followed by an interventional one arm clinical trial design (quasi experiment) were performed to fulfill the preset objectives. The case-control study included 89 prediabetic patients with peripheral neuropathy (Group A) and 89 prediabetics without peripheral neuropathy (Group B).

Study Participants and Case Definition: Prediabetic individuals were diagnosed, according to the American Diabetes Association 2019, with impaired fasting (100–125 mg/dl) and/or impaired glucose tolerance (140–199 mg/dl), and/or glycated haemoglobin (5.7–6.4%)⁶ (they were all ages between 18–60 years old). Participants were recruited from the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt, in the period from September 2018 to March 2019 after proven informed written consent.

Ethical Considerations: A written informed consent was obtained from all patients. All data were made confidential and an ethical approval on study conduction was obtained from the Local Research Ethical Committee (REC) of the Faculty of Medicine, Ain Shams University. FWA 000017858.

All participants were subjected to full medical history including smoking habits, alcohol consumption, drug history, thorough clinical examination including blood pressure, weight, height and BMI.

Screening for peripheral neuropathy

All participants were screened for peripheral neuropathy by 10 g monofilament for assessing the loss of protective

sensation, tuning fork (vibration sense testing using a 128-Hz tuning fork), ankle reflex, pinprick (for perception of pain) and Douleur Neuropathic 4 diagnostic questionnaire (DN4)⁷ that assesses symptoms reflecting pain in the form of burning, painful, cold, electric shocks, tingling, pins and needles. If the patients score is ≥ 4 the patient likely suffers from neuropathic pain. Patients found to have peripheral neuropathy were given the Short-Form McGill Pain Questionnaire (SF-MPQ)⁸ that assesses the severity of pain; an increase in the score indicates increasing severity.

Laboratory studies

Laboratory studies included: ionized calcium, phosphorous, PTH, and 25(OH)vitamin D, FBG, 2h-PPBG, glycated hemoglobin and total lipid profile (total cholesterol, low density lipoprotein (LDL) and triglycerides)

Participants were first instructed to fast for eight hours (overnight fasting), 10 ml of venous blood were then collected by venipuncture without tourniquet. 2 ml of the collected blood were taken in an EDTA containing tube for the assay of the glycated hemoglobin and it was stored at 4°C to be carried out within one week. 2 ml were taken in a fluoride containing tube and then separated by centrifugation and the sample was used for measurement of FBG, serum Ca, phosphorus, PTH and 25(OH)vitamin D. 2 ml sample were collected two hours after 75 g oral glucose load for the measurement of the 2h-PPBG. On a separate day, 2 ml of venous blood were collected by venipuncture (after an overnight 12 hour fast), the sample was collected in a fluoride containing tube and then separated by centrifugation and used for measurement of total lipid profile (total cholesterol, low density lipoprotein (LDL), triglycerides (TG)) by enzyme colorimetric assay. Total cholesterol level was measured by Quantitative Enzymatic-Colorimetric assay (Catalogue Number: 1010/ manufacturer: Stanbio-Laboratory, Inc., USA/ Boerne, Texas/ 1/2018). Triglyceride level was measured by Quantitative Enzymatic-Colorimetric assay (Stanbio LiquiColor Triglycerides/ Catalog Number: 2100/ manufacturer: Stanbio-Laboratory, Inc., USA/ Boerne, Texas, USA/ 03/2018). LDL cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant (HDL and VLDL) after precipitation of LDL fraction by polyvinyl sulphate in the presence of polyethylene-glycol monomethyl ether. Calculation $LDL = \text{Cholesterol} - (\text{HDL} + \text{Triglyceride}/5)$. HDL level was measured by Quantitative Enzymatic-Colorimetric assay (Stanbio HDL cholesterol/ Catalog Number: 0599/ manufacturer: Stanbio-Laboratory, Inc., USA/ Boerne, Texas, USA/ 02/2018). Serum 25-hydroxyvitamin D level was measured by an ELISA kit, which is a solid phase enzyme-linked immunosorbent assay (ELISA, Catalogue Number: 10501, Chemux Bioscience, Inc., Hayward, CA/ 10/2018). Parathormone level was measured by an ELISA kit with a normal range of 10–55 pg/ml (ELISA, Catalogue Number: KAP1481, DIAsource ImmunoAssays S.A, Nivelles, Belgium/ 2/2018). Glycated hemoglobin was measured by quantitative colorimetric determination of glycated haemoglobin in whole blood (Catalog Number: 0350/ manufacturer: Stanbio-Laboratory, Inc., Boerne, Texas, USA/ 06/2018). Fasting blood glucose, 2h-PPBG were measured by Stanbio Glucose LiquiColor

(Oxidase) (Catalog Number: 1070, manufacturer: Stanbio-Laboratory, Inc., USA, Boerne, Texas, USA/ 04/2018). Vitamin D status was assessed according to Hovsepian *et al.*, and classified as Sufficient if higher than 30ng/ml, Insufficient if between 20–29 ng/ml and Deficient if less than 20 ng/ml⁹.

Exclusion criteria

Patients with renal impairment, hypo or hyperthyroidism, patients on vitamin D supplementation or antiepileptic or any medication affecting calcium and vitamin D level, pregnant or breast-feeding females were excluded from the study.

Based on the results of previous studies, the majority of patients with and without peripheral neuropathy were found to be either insufficient or deficient as regard their level of vitamin D. The current analytical study has also demonstrated that all prediabetics with and without peripheral neuropathy were also either insufficient or deficient as regard their level of vitamin D.

Interventional Study: Patients of group A were invited to participate in a quasi-experiment by administration of therapeutic dose of vitamin D. None of the 89 patients participating in the case-control study refused to be enrolled in the interventional study. 200,000 IU of Vitamin D (cholecalciferol) were intramuscularly administered every month for three successive months to all patient with peripheral neuropathy.

Clinical assessments were repeated in the last visit after three months to assess the improvement in peripheral neuropathy in those patients. Retesting is advised after three months, as suppression of parathyroid hormone after supplementation with cholecalciferol takes at least three months and the response differs between individuals. So, most guidelines recommend repeat testing after three months¹⁰.

All laboratory tests were conducted at the beginning of the study and after three months of vitamin D supplementation.

Statistical analysis

Collected data were analyzed using SPSS (version 17, 2012, IBM Corporation, USA) (An open-access alternative that can perform an equivalent function is the R Stats package). The continuous quantitative variables included Age (Years), BMI (kg/m²), Systolic BP (mmHg), Diastolic BP (mmHg), HbA1c (%), 2h-PPBG (mg/dl), T. cholesterol (mg/dl), LDL (mg/dl), HDL (mg/dl), TG (mg/dl), 25 (OH) Vit D (ng/ml), Ionized Ca (mg/dl), Phosphorus (mg/dl), PTH (pg/ml) and they were described as mean and standard deviation. Student's T-test was used to compare two independent groups (group A and group B) for quantitative data. Continuous variables before and after vitamin D intake were assessed using paired t-test. Regarding categorical/qualitative data, we measured vitamin D status (Sufficient, Insufficient, Deficient) and they were presented as numbers and percentages and the Chi-Square test was used to compare two independent groups (group A and group B) with qualitative data. Additional qualitative data that were measured were clinical examination for peripheral neuropathy using ankle reflex, tuning fork (vibration) and 10 g monofilament

and they were presented as numbers and percentages and McNemar and McNemar Bowker tests were used to compared group A before and after vitamin D supplementation.

Results

Comparison between the two studied groups regarding clinical and laboratory characteristics is shown in **Table 1**. 20.2% of patients in group A (With PN) reported vitamin D insufficiency (N=18), while deficiency was evident in 79.8% (N=71) and none reported normal level of vitamin D. While group B

(Without PN): 9 (10.1%) were insufficient, 80 (89.9%) were deficient and none were sufficient (**Table 2**); with a non-significant difference in the mean level of vitamin D level between the two groups (13.957 ± 6.3603 ng/ml (group A) vs 14.594 ± 3.9318 ng/mL (group B) ($P>0.05$) (**Table 1**).

Vitamin D level showed a negative but statistically insignificant correlation with the severity of peripheral neuropathy (assessed by SF-MPQ) ($r= 0.032$, $p= 0.766$) and pain score (assessed by DN4 questionnaire) ($r= 0.052$, $p= 0.629$)

Table 1. Comparison between the two studied prediabetic patient with (Group A) and without (Group B) peripheral Neuropathy.

	Group(A) (N=89)	Group (B) (N=89)	Sig	Student t test
	Mean±SD	Mean±SD	P-value	T-test
Age (Years)	50.416±7.9841	42.876±10.4183	≤0.001	-5.419
BMI (kg/m ²)	30.416±2.9573	30.236±3.2193	0.699	-0.388
Systolic BP (mmHg)	131.52±10.068	129.82±10.784	0.28	-1.08
Diastolic BP (mmHg)	80.28±9.925	74.38±8.147	≤0.001	-4.33
HbA1c (%)	5.94±0.20	5.91±0.22	0.353	-0.931
FBG (mg/dl)	101.92±15.28	92.02±12.00	≤0.001	-4.806
2h-PPBG (mg/dl)	150.04±26.18	121.75±29.92	≤0.001	-6.713
T.cholesterol (mg/dl)	197.730±25.55	191.32±30.04	0.127	-1.532
LDL (mg/dl)	135.00±19.76	112.48±29.37	≤0.001	-6.000
HDL (mg/dl)	43.70±12.70	55.70±15.10	≤0.001	5.7
TG (mg/dl)	112.58±27.88	115.12±21.22	0.495	0.684
25 (OH) Vit D (ng/ml)	13.95±6.36	14.59±3.93	0.423	0.804
Ionized Ca (mg/dl)	4.591±0.4220	4.244±0.5114	≤0.001	-4.940
Phosphorus (mg/dl)	3.434±0.1167	3.582±0.2203	≤0.001	5.612
PTH (pg/ml)	80.22±18.07	72.34±16.67	≤0.05	-3.021

BMI= body mass index, BP= blood pressure, HbA1c = glycated haemoglobin, 2h-PPBG = 2 hour post 75g glucose, T.cholesterol = total cholesterol, LDL = low density lipoprotein, , HDL = high density lipoprotein, TG = serum triglycerides, 25 (OH) Vit D= 25 hydroxy Vitamin D, Ionized Ca= ionized calcium, PTH = serum parathyroid hormone.

After vitamin D supplementation

There was a highly significant improvement in vitamin D level in group (A) after intramuscular injection of vitamin D, from which 42 (47%) prediabetic patients became sufficient, 38 (42.7%) became insufficient and only 9 (10.1%) remained deficient ($P \leq 0.001$). There was a highly significant improvement of glycemic profile as shown in (Table 3).

Neuropathic pain score and its severity

A statistically significant improvement in neuropathic pain severity was observed by the SF-MPQ (28.3 ± 7.2 before and 17 ± 6.3 after vitamin D supplementation, $P \leq 0.001$). There was also a statistically significant reduction in the DN4 questionnaire score from 6.39 ± 1.64 to 2.5 ± 0.9 , ≤ 0.001 (≥ 4 denote neuropathic pain) with an improvement of neuropathic pain in 82% of patients (73 out of 89) ($P \leq 0.001$) (Figure 1).

Clinical examination for peripheral neuropathy

We found a highly significant improvement in vibration sense by tuning fork and protective sense measured by the 10 g monofilament test ($P \leq 0.001$), while there was no improvement regarding ankle reflex ($P > 0.05$) (Table 4).

Discussion

Diabetic peripheral neuropathy in recently diagnosed diabetic patients may reach about 8% and more than 50% in patients

with long-standing diabetes¹¹. Recently, the American Diabetes Association stated that there is no strong evidence that supports the lifestyle management or efficacy of glycemic control in the treatment of neuropathic pain, which means that pharmaceutical interventions such as pregabalin, duloxetine, or tapentadol are the only way of treatment¹². Accordingly, we aimed to demonstrate the association of vitamin D status with peripheral neuropathy and determine the effect of vitamin D supplementation on painful neuropathy in prediabetics.

Even with our sunny country, none of our patients (0%) had sufficient vitamin D level.

Kuchay *et al.*, 2015 in their study found that prediabetes patients were 54.3% vitamin D deficient, 21.3% were insufficient and only 24.4% were sufficient despite abundant sunshine in India¹³.

Kuchay *et al.*, (2015) demonstrated an association between vitamin D status and prevalence of diabetes, with low prevalence in people with high vitamin D status and a belief that a serum 25(OH) vitamin D level of 15 ng/mL or less may be a threshold at which vitamin D deficiency confers negative effect on insulin sensitivity¹³. This was confirmed when nearly 50% of patients with prediabetes had serum 25(OH) vitamin D levels below 15 ng/mL¹³. On the contrary, Rolim *et al.*, (2016) found

Table 2. Vitamin D status among the studied groups.

	Vitamin D status						Total		P-value
	Sufficient		Insufficient		Deficient		Number	%	
	Number	%	Number	%	Number	%			
Group (A)	0	0%	18	20.2	71	79.8	89	100	0.095 NS
Group (B)	0	0%	9	10.1	80	89.9	89	100	
Total	0	0%	27	15	151	85	178	100	

*Group A= Prediabetic patients with peripheral neuropathy

Group B= Prediabetic patients without peripheral neuropathy

Table 3. Comparison of prediabetic patients with PN before and after Vitamin D Supplementation (Group A).

	Group (A) before vitamin D (N=89)	Group (A) after vitamin D (N=89)	Sig.	Paired t test
	Mean±SD	Mean±SD	P-value	t-test
HbA1C (%)	5.94±0.20	5.707±0.33	≤0.001	7.45
FBG (mg/dl)	101.921±15.2831	92.258±15.2722	≤0.001	6.203
2h-75g glucose (mg/dl)	150.045±26.1816	102.000±16.7359	≤0.001	17.309

*HbA1c = glycated haemoglobin, FBG= fasting blood glucose, 2h-75g glucose=2-hour post 75g glucose

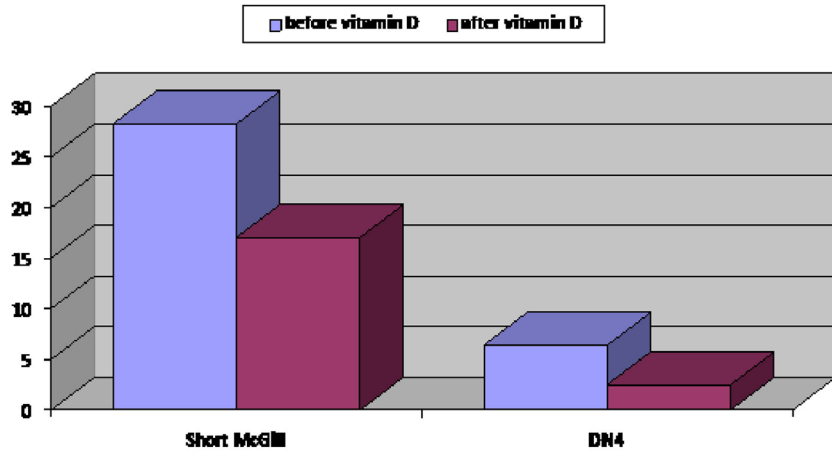


Figure 1. Neuropathic pain score assessed by Douleur Neuropathic 4 score and severity of peripheral neuropathy assessed by the Short-Form McGill Pain Questionnaire score of group A (prediabetic patients with peripheral neuropathy) before and after vitamin D supplementation.

Table 4. Peripheral neuropathy indicators (ankle reflex, tuning fork vibration and 10 g monofilament) in group A (Prediabetics with PN) before and after vitamin D injection.

Before	After			MNB
	Ankle reflex			P value
Ankle reflex	Absent N (%)	Present N (%)		NS
Absent	1 (100.0)	0 (0.0)		
Present	0 (0.0)	88 (100.0)		
	Vibration			47.0 <0.001
Vibration	Absent N (%)	Reduced N (%)	Present N (%)	
Absent	2 (100.0)	29 (85.3)	12 (22.6)	
Reduced	0 (0.0)	5 (14.7)	6 (11.3)	
Present	0 (0.0)	0 (0.0)	35 (66.0)	
	Monofilament After			68.0 <0.001
Monofilament	Absent N (%)	Reduced N (%)	Present N (%)	
Absent	6 (100.0)	35 (92.1)	27 (60.0)	
Normal	0 (0.0)	0 (0.0)	12 (26.7)	
Reduced	0 (0.0)	3 (7.9)	6 (13.3)	

MNB=McNemar Bowker test between patients with peripheral neuropathy before and after vitamin D injection

Paired t-test in both Short McGill and DN4 <0.001

the association between HbA1c and 25(OH) vitamin D controversial and glycemic control was not associated with vitamin D level¹⁴. Luo *et al.*, (2009) stated that there was no impact of hypovitaminosis D on metabolic syndrome status and HbA1c¹⁵.

The association between vitamin D status and prevalence of diabetes can be explained through the effect of vitamin D on

pancreatic β -cell function and plasma calcium. Vitamin D deficiency decreases serum calcium, which regulates insulin synthesis and release¹⁶.

On the other hand, administration of vitamin D causes increase in serum calcium, decrease in circulating free fatty acid levels, increase in insulin release and improvement in glucose levels¹⁷.

Shehab *et al.*, (2012) study on 210 diabetic patients, from which 87 had peripheral neuropathy, first found that vitamin D deficiency was significantly associated with diabetic peripheral neuropathy¹⁸. In agreement with Shillo *et al.*, (2019) who reported that serum vitamin D levels were lower in patients with painful DPN than in those with painless DPN, and pain scores were negatively correlated with serum vitamin D levels¹⁹.

On the contrary, Basit *et al.*, (2016) acknowledged that there was no significant correlation between 25 (OH) vitamin D status with either total McGill pain location, McGill pain score, DN4 or positive symptoms²⁰ and this was inconsistent with the present study. Studies by Usluogullari *et al.*, (2015) also found no difference in the prevalence of vitamin D deficiency between diabetic peripheral neuropathy patients and controls²¹.

However, Shehab *et al.*, (2012) study confirmed that vitamin D was the only independent risk factor for diabetic peripheral neuropathy¹⁸. While in China, He *et al.*, (2017) declared that deficiency of vitamin D is an independent risk factor for diabetic peripheral neuropathy and can be considered a potential biomarker for peripheral neuropathy in diabetic Chinese patients²².

On the other hand, Alkhatatbeh *et al.*, (2019) showed that the only significant predictor for neuropathic pain was female gender, while vitamin D level, BMI, age, FBG, duration of T2DM, DBP and SBP were not²³. The divergence in the results of previous studies may be due to the use of different methods to assess neuropathy and because the studies were directed on different populations.

Injection of vitamin D 200.000 IU intramuscular every month for three successive months is in accordance with the guidelines for vitamin D supplementation and treatment of deficiency in Central Europe individuals with proved vitamin D deficiency which require higher doses of vitamin D than doses recommended for the general population. The therapeutic dose in severe deficiency should be 1.000–10.000 IU/day (~50.000 IU/week), depending on the patient's body weight and age. The duration of the treatment varies from 1–3 months, depending on the degree of vitamin D deficiency²⁴. Our patients showed significant improvement and reduction in neuropathy severity score and also showed clinical improvement by monofilament and tuning fork. This is in line with Bell (2012) who found great improvement in neuropathic symptoms after supplementation with 50.000 IU of vitamin D₂ every week in a case report of a patient suffering from diabetic peripheral neuropathy. The patient had been refractory to different types of treatment like tricyclic's, gabapentin, oxycodone and pregabalin²⁵. As well, Shehab *et al.*, (2015) in their study applied vitamin D replacement therapy as a single intramuscular vitamin D dose of 300.000 IU and this application significantly enhanced the DN4 questionnaire scores of the patients with diabetic neuropathy²⁶. Correspondingly, Lee and Chen (2008) showed that oral cholecalciferol resulted in an approximate 50% reduction in painful neuropathic symptoms and a significant reduction in SF-MPQ score from 32.1 to 19.4; however, this study had neither a placebo group nor was randomized, leaving it open to considerable bias²⁷.

Possible explanation of previous studies was demonstrated *in vitro* by Fukuoka *et al.*, (2001) and *in vivo* by Riaz *et al.*, (1999) who considered vitamin D as a neurotrophic substance, which modulates neuronal growth and differentiation, and neuromuscular functions^{28,29}. Its exact role in diabetic neuropathic pain is uncertain; insufficiency of vitamin D may increase damage of diabetic nerve and may affect the function of nociceptors leading to pain at a higher threshold of serum 25 (OH) vitamin D concentration higher than that in the non-diabetic individuals²⁷.

Therefore, the results of previous studies corroborate our findings that vitamin D supplementation improves peripheral neuropathy and can be used as a safe treatment for peripheral neuropathy in prediabetic patients with hypovitaminosis D.

Opposing previous results, a study by Alam *et al.*, (2016) reported no significant decrease in neuropathic pain scores after vitamin D administration³⁰. This study was based on all or none values instead of assessing the quantity of pain score, which may have led to a failure of observing a reduction in pain scoring.

Glycemic parameters of our patients showed significant improvement after the administration of 200.000 IU of vitamin D every four weeks for 12 weeks, which was the same result found by Kuchay *et al.*, (2015) who revealed that correcting vitamin D deficiency in people with prediabetes significantly reduces FBG, two hours plasma glucose and A1C levels in 12 months¹³. However, contrary to our findings, He *et al.*, (2018) proclaimed in their meta-analysis that vitamin D supplementation did not improve fasting glucose levels or insulin resistance, nor did it prevent T2DM in non-diabetics³¹. Furthermore, Moreira-Lucas *et al.*, (2017) confirmed that vitamin D supplementation did not improve fasting or post challenge measures of insulin sensitivity, β -cell function or HbA1c³².

Among the limitations of the study were a small sample size compared to previous studies. Our study is the first to discuss the effect of vitamin D supplementation on peripheral neuropathy in prediabetic individuals whereas other studies have discussed the effect on diabetic patients. Finding prediabetic participants with peripheral neuropathy to include in the study was challenging.

Conclusion

This study found that vitamin D supplementation in prediabetics with peripheral neuropathy and hypovitaminosis D improves neuropathy in those patients as assessed by McGill and DN4 scores, as well as glycemic parameter namely HbA1c, FBG and 2h-PPG.

Data availability

Underlying data

Figshare: Underlying data for 'The impact of vitamin D supplementation on peripheral neuropathy in a sample of Egyptian prediabetic individuals', <https://doi.org/10.6084/m9.figshare.16831858>³³

This project contains the following underlying data:

- Data file 1: prediabetic patients without peripheral neuropathy and their descriptive and laboratory data.
- Data file 2: prediabetic with peripheral neuropathy and their descriptive, laboratory data, McGill Pain Questionnaire, clinical examination for neuropathy before vitamin D supplementation.

- Data file 3: prediabetic with peripheral neuropathy and their descriptive and laboratory data, McGill Pain Questionnaire, clinical examination for neuropathy after Vitamin D supplementation.

Consent statement

Written informed consent was obtained from all individual participants included in our study.

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Ghada M. El Sagheer

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Thanks to the authors for their choice of this subject of study which appears to be interesting and not frequently discussed before. It's a really well-organized study, well-constructed title, discussing the effect of vitamin D supplementation on prediabetic individuals which is the first study so far discussing this specific point

The introduction was clear and updated, showing that microvascular complications like peripheral neuropathy can occur before the development of diabetes (Nebuchennykh M. *et al.*, 2008¹) and highlighting the association between peripheral neuropathy and vitamin D (Qu GB *et al.*, 2017²).

The methodology was perfectly planned, well written, and well-arranged so that the reader can understand the steps of the study and how the authors managed to find prediabetic individuals with peripheral neuropathy. Also, the number of patients is very suitable to the study design, helping to get more valid data on the effect of vitamin D supplementation on peripheral neuropathy as previous studies were lacking evidence due to either small sample size or defect in follow-up.

The statistics are well prepared, the sample size is quite enough for the study and the data is analyzed by SPSS. The statistical analysis and tests are properly described and appropriate for the study, used correctly which helped to get the results that are interpreted in a clear way. The tables and figures are well presented and have a crucial role in the study and writing of the manuscript and helped to finalize the results of the study. Finally, data files are uploaded which will help produce more results further on it, but it would be much better to present the raw data in a simplified way.

The results were nicely presented; the authors first presented that vitamin D deficiency was highly prevalent in the studied group. Second, the authors compared clinical and laboratory data between the two studied groups. Third, they presented the effect of vitamin D supplementation over the arm with the peripheral neuropathy and clarified the benefit on the vitamin D levels and neuropathy score and severity and clinically by peripheral neuropathy indicators (ankle reflex,

tuning fork vibration, and 10 g monofilament).

The discussion was organized and updated, citing each part of the study with other papers, discussing with cons, and clarifying the cause of each result.

The conclusion was short, to the point, summarizing all the aims in the study design.

Despite this, I would be thankful if the authors could clarify the following points:

1. The main results are not sufficiently presented in the abstract (e.g. the mean and SD of the vitamin D level in the study groups, the mean of HbA1c results, and the other glucose parameters, etc).
2. In the methods section, I need to know whether the pre-diabetic individuals received any management, e.g. lifestyle modifications, exercise, metformin, to clarify that neuropathy improvement is not related to other factors.
3. What are the definitions of vitamin D deficiency, insufficiency, and sufficiency the authors used, and which reference?
4. In the results sections, the mean level of vitamin D of the studied groups was not written in any part of the results or tables.

Finally, I find this study accepted, well-organized, and well-written.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endocrine disorders

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 24 January 2022

<https://doi.org/10.5256/f1000research.78627.r101602>

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Zaynab Alourfi

Faculty of Medicine, Damascus University, Damascus, Syria

Ibrahim Alali

Damascus University, Damascus, Syria

This article discusses a common health problem facing diabetologists and internal medicine professionals.

- The authors have properly introduced the aim of the study in the introduction, especially the fourth paragraph. For instance, a lot of papers (Xiaohua G *et al.*, 2021¹ and Shehab D *et al.*, 2012²) talked about Vit D deficiency in relation to diabetic neuropathy, and in clinical practice a lot of Vit D prescriptions are without clear evidence, but above that, no data about prediabetes, so focusing on this point is great.
- The methodology was clear after the latest revision and helps to reproduce results by other researchers.
- I prefer that the 75 g glucose tolerance test be referred to with "75g-OGTT" rather than "2h-PPBG" in the laboratory studies and other places.
- Regarding statistical analysis, the authors described statistical tests properly but to give an accurate criticism, the reviewer should be more specialized in statistics. Data files are uploaded completely and allow who wants to use them in reproducing results.
- Results were well described and organized; authors started with sample characteristics and organized them in Table 1 properly, then moved to Vit D results and followed up with results after replacement, then described neuropathic pain scores and also organized it in tables.
- The discussion was very good, citing the literature and comparing results to other papers was well established, and I really appreciate the authors' comment about how hard it is to

find patients with such inclusion criteria given my expertise. The authors declared that one limitation was collecting patients compatible with the inclusion criteria, it's really challenging to find a prediabetic patient accept, confirming the diagnosis by fasting and doing OGTT test then assessment of neuropathic pain, so this effort from the authors is really appreciated in my opinion.

- The conclusion was short but I think it is enough to support the results since it is mentioning the primary outcome for this paper which is that replacement of Vit D in neuropathic prediabetes patients is useful.

Finally, I find this version accepted and well written.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vitamin D disorders, osteoporosis, hypothyroidism, diabetes

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Comments on this article

Version 1

Author Response 17 Aug 2021

naira hany khalil, Ain Shams University, Cairo, Egypt

Thank you for your kind reply. A sample size of 175 cases of prediabetics was calculated using Epi Info™⁷ program using prevalence of vitamin D deficiency among prediabetics = $(87 \pm 5) \%$ with accepted range $(82-92) \%$ at 95% C.I.¹¹

And then all participants were screened for peripheral neuropathy by 10 g monofilament for assessing the loss of protective sensation, tuning fork (vibration sense testing using a 128-Hz tuning fork), ankle reflex, pinprick (for perception of pain) and Douleur Neuropathic 4 diagnostic questionnaire (DN4)⁷ that assesses symptoms reflecting pain in the form of burning, painful, cold, electric shocks, tingling, pins and needles. If the patient's score is ≥ 4 the patient likely suffers from neuropathic pain. Patients found to have peripheral neuropathy were given the Short-Form McGill Pain Questionnaire (SF-MPQ)⁸ that assesses the severity of pain; an increase in the score indicates increasing severity.

And then we divided them into 89 participants with peripheral neuropathy according to clinical examination and DN4 pain questionnaire and 89 participants without neuropathy according to the same qualifications.

Competing Interests: none

Reader Comment 16 Aug 2021

Mohammad Ali, Uttara Adhunik Medical College and Hospital, Dhaka, Bangladesh

This is an important study, however, the method looks confusing. It is not clear how you create Group A and B.

Competing Interests: None

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