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RESEARCH ARTICLE

Effects of vitamin D3 (cholecalciferol) supplementation on diabetic polyneuropathy in patients diagnosed with diabetes mellitus

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Introduction: Peripheral sensorimotor polyneuropathy is present in nearly half of the patients diagnosed with diabetes mellitus. Over the past 10 years, animal and human studies have suggested that vitamin D3 treatment may have a role in preventing or reducing neuropathic complaints and symptoms. **Material and method**: Our clinical, prospective, interventional, placebo-controlled study investigated the therapeutic effect of 2.000 IU oral cholecalciferol administered for three months on diabetic polyneuropathy. Patients treated with vitamin D and B, thioctic acid, and other analgesics were excluded. Using the single-blind technique, they were randomly assigned into vitamin D-treated and placebo-treated groups. In addition to recording anamnestic data, the study included - a Toronto Clinical Neuropathy Scoring System and Michigan Neuropathy Screening Instrument based - questionnaire to assess subjective symptoms and a physical examination including sensory tests (fine touch-, temperature awareness, pain-, vibration perception). Vitamin D levels were measured. After three months of therapy, the examination was repeated. **Results**: Most of the patients were found to have vitamin D deficiency (36% of the total population) or insufficiency (43%). In the cholecalciferol-treated group, but not in the control group, subjective symptoms decreased in intensity and/or frequency, and a significant improvement in the overall complaint scale was observed ($p = 0.006$), but no change regarding the sensory tests ($p > 0.05$). **Conclusions**: Our results show that oral administration of cholecalciferol for three months significantly reduced subjective symptoms and neuropathic pain as assessed by our questionnaire, however, there was no significant change in the results of the sensory tests. Vitamin D deficiency/insufficiency was common in diabetic patients (79% in our population), therefore screening is recommended.

Keywords: diabetes mellitus, cholecalciferol, diabetic polyneuropathy, neuropathic pain

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Introduction

Peripheral sensorimotor polyneuropathy (PSMN) is present in nearly half of the patients diagnosed with diabetes mellitus. The most common form of diabetic neuropathy is chronic peripheral sensorimotor polyneuropathy (about 75%), characterized by distal, symmetrical sensory and motor loss in the limbs, which appears as paresthesia (burning feet), neuropathic pain, allodynia (brush-evoked), areflexia, and later motor disturbances [1]. Distal, symmetric sensorimotor polyneuropathy can be divided into three types of lesion: dominant small fiber, dominant large fiber, and mixed (the most common form). In dominant small fiber neuropathy, the perception of temperature and pain is affected, whereas, in large fiber neuropathy, the sensation of stability (abnormal proprioception), fine touch, and vibration perception are impaired [1,2].

Diabetic polyneuropathy is a neurodegenerative disease with several factors involved in its pathophysiological mechanism. Increased oxidative stress promotes the activation of certain abnormal metabolic pathways such as the polyol and hexosamine pathways and advanced glycation end products (AGEs), poly-ADP-ribose polymerase, and protein kinase C, which damage the tissue structure of the nerve, negatively affecting its function [3]. Microangiopathy, i.e. abnormal changes in the capillaries that negatively affect the blood supply to peripheral nerves, also plays an important role in the pathophysiological mechanism of diabetic neuropathy [4].

According to an international consensus published in 2021, the positive clinical diagnosis of diabetic sensorimotor polyneuropathy is confirmed by the bilateral presence of reduced vibration and/or pain perception, and the clinically characteristic pain in the same location. The treatment of diabetic sensorimotor polyneuropathy includes adequate glycemic control, prevention of cardiovascular risk factors, pathogenetically oriented pharmacotherapy (alpha-lipoic acid, benfotiamine), symptomatic treatment of neuropathic pain with pharmacological and non-pharmacological analgesics [5].

The "classic" effects of vitamin D are regulation of calcium absorption, independent effects on bone and other tissues, and regulation of PTH and calcitriol production through negative feedback [6]. In addition to these effects, vitamin D is also known to have immunomodulatory effects; a correlation between autoimmune diseases and vitamin D levels [7], and effects on B and T lymphocyte function were found [8,9]. Vitamin D also has antioxidant effects, reducing oxidative stress by inhibiting various metabolic pathways and the accumulation of its end products [6]. In addition, it improves microcirculation and has anti-inflammatory effects (reducing IL-6 levels and in-

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creasing IL-10 levels) [10]. A neuroprotective effect was also demonstrated, which makes vitamin D deficiency one of the factors in the development of various neurological pathologies [11].

Several studies have investigated the effect of vitamin D or its deficiency on neuropathic pain. In a preclinical study, artificially induced vitamin D deficiency in mice caused chronic pain and microglial lesions [11]. Clinical studies have observed that oral administration of vitamin D significantly reduced the severity of polyneuropathy and neuropathic pain [10]. Intramuscular administration also significantly reduced neuropathic pain and paresthesia [12]. Mechanisms involved in the effect of vitamin D on neuropathic pain include inhibition of COX-2 expression, stimulation of 15-prostaglandin dehydrogenase (PGDH), upregulation of neurotrophin (NT) synthesis, suppression of tumor necrosis factor-alpha (TNF-alpha) and macrophage colony-stimulating factor (M-CSF) in astrocytes and microglia, attenuation of the decrease in antinociception markers, prevention of the increase in lipid hydroperoxide levels, superoxide anion generation (SAG) and hydrogen peroxide (H2O2), prevention of the decrease in total thiol content, increase in total antioxidant capacity (TAC) [13,14].

Material and method

Our clinical trial was a prospective, interventional, placebo-controlled study to investigate the therapeutic effect of oral vitamin D_3 supplementation, administered for three months in patients with diabetic polyneuropathy. We also mapped the prevalence of vitamin D deficiency in patients with diabetic polyneuropathy.

To be eligible for the therapy used in the research, the subjects had to meet the following criteria: diagnosed with type 1 or type 2 diabetes mellitus, diabetic sensorimotor peripheral polyneuropathy, adequate cooperation, and signed informed consent. Exclusion criteria were: had received one of the following treatments for at least one full month in the three months before the study: vitamin D, vitamin B, thioctic acid, antiepileptic, antituberculotic, had a SARS-CoV-2 infection in the three months before the study, had a proven history of the polyneuropathy of other etiology, had a proven history of leg pain of other etiology, previous amputation of a lower limb, hyperthyroidism, hyperparathyroidism, hypercalcemia, renal insufficiency, chronic alcoholism, psychiatric illness, HIV infection or hepatitis B, C infection, inadequate cooperation.

Our research was conducted in a Diabetes Day Care Center in Târgu Mures, between October 2021 and June 2022. The study was divided into three main parts. In the first part, we collected data including the patient's gender, age, duration of diabetes, medication, regularity of medication, presence of comorbidities (related to exclusion criteria), weight, and height. The laboratory findings collected were glycated hemoglobin (HbA1c), serum vitamin D (25-OH-D), and serum creatinine. Body Mass Index was calculated from body weight and height using the formula BMI = m/h^2 (m = body weight, h = height in meters). The glomerular filtration rate (GFR) was calculated from serum creatinine using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation adjusting for sex, age, and race. Enzyme-linked fluorescent assay (ELFA) and electrochemiluminescence immunoassay analyzer (ECLIA) were the methods we used for the measurement of serum vitamin D levels (25-OH-D), and vitamin D deficiency was defined as values below 20 μg/l and vitamin D insufficiency as values between 20-30 μg/l. Serum concentrations above 30 μg/l were considered normal.

Secondly, we performed a 15 closed questions assessment, to evaluate the subjective symptoms of peripheral polyneuropathy. We used a bilingual questionnaire (Romanian and Hungarian) based on the Toronto Clinical Neuropathy Scoring System [15] and the Michigan Neuropathy Screening Instrument (MNSI) [16,17]. The maximum score for the questionnaire was 13, as each question was scored as 1 or 0 and two questions were not scored. High probability for PSMN was considered with a questionnaire score above 2 points.

In the third part we performed a physical examination, including sensory tests, where pain perception (prick test), temperature perception (tip therm test), vibration perception, and fine touch (monofilament test) were measured. The score of the sensory tests was as follows: 0 points if no pathological changes were detected during the test, 1 point if abnormalities in the function were detected, and 2 points if a complete absence of the function was observed. The sensory tests were performed on both lower limbs and if the score showed a discrepancy between the two lower limbs affected in a given function, the higher score of the patient was taken into account. The presence of neuropathic pain was assigned with a score of 1, and the absence with a score of 0.

PSMN was confirmed if: the score of the questionnaire was above 2 and/or 2 sensory tests showed modified parameters. Once we had completed our three-part examination and the patient met the inclusion criteria, he was included in one of the two groups using a randomized, single-blind technique. Patients in the treatment group took a dietary supplement containing 2.000 IU doses of vitamin D_3 oral tablet once per day for 90 days. Members of the placebo group also received treatment for 90 days, during which they took a tablet of the same phenotype without an active ingredient, also taken orally one per day. The placebo tablets were produced by the Department of Pharmaceutical Technologies at the George Emil Palade University of Medicine, Pharmacy, Science and Technology in Târgu Mures. After 90 days, the treatment group and the control group were re-examined.

The study was approved by the Ethical Committee of the George Emil Palade University of Medicine, Pharmacy, Science and Technology from Târgu Mures, the registration number of the decision is 1484/2021.10.27.

The data were summarized in a Microsoft Excel spreadsheet. After creating the database, we used IBM SPPS 20 to perform our statistical analysis. We used independent T-test or paired T-test for parametric distributed, quantitative, and numerical data, and Mann-Whitney U test or Wilcoxon test for non-parametric distributed, qualitative, and ordinal data. The results were considered significant if $p < 0.05$.

Results

In our study, 360 diabetic patients were screened, of which 47 were found eligible for the full examination. After completion of the examination, 41 subjects were recruited into the study, of which 23 subjects were included in the treatment group and 18 subjects in the placebo control group. Results were considered significant if $p < 0.05$.

Characteristics at baseline

The major risk factors for PSMN include a longer duration of diabetes and high glycated hemoglobin levels, but it may be also associated with various elements of the metabolic syndrome, especially in type 2 diabetes, such as high body mass index (BMI) [20]. These risk factors were also present in most of our study patients, although we did not investigate the possible causal link between these two. The mean duration of diabetes in our study subjects was 15.91 ± 8.85 vs. 16.06 ± 10.12 years, and the mean body mass index was 29.46 ± 5.45 vs. 30.14 ± 5.31 kg/m^2. Regarding the BMI, 12.5% of the patients had normal weight, 42.5% were overweight, 30% had grade 1 obesity, and 15% grade 2 obesity. In terms of glycated hemoglobin levels, 44% of our study subjects had values below 7%, 44% between 7-8%, while 12% had values above 8%. No significant difference was found between the two groups for these initial characteristics ($p > 0.05$).

In terms of serum vitamin D concentrations, the mean value of the groups did not differ significantly (24.71 ± 8.16 vs. 14.93 ± 5.85, $p = 0.073$), but the prevalence of vitamin D insufficiency/deficiency in the study population was high, as it is shown in *Figure 1.*

We found that the mean of the questionnaire scores was almost the same in the two groups. The prevalence of neuropathic pain among our subjects exceeded 50% in both groups, (65.2% vs. 55.5%, $p = 0.748$). The difference in pain-, temperature-, vibration perception and fine touch impairment did not reach a statistically significant level between the two groups. These results are summarized in *Table 1.*

Change in parameters after vitamin D_3 treatment

The subjective symptoms assessed by the questionnaire were significantly decreased after therapy at the three-month follow-up in the treatment group (4.43 ± 1.61 vs. 3.74 ± 1.73, $p = 0.006$), but not in the placebo group (4.17 ± 1.38 vs. 3.94 ± 1.3, $p = 0.157$). In terms of neuropathic pain prevalence, the initial prevalence rate of 65%

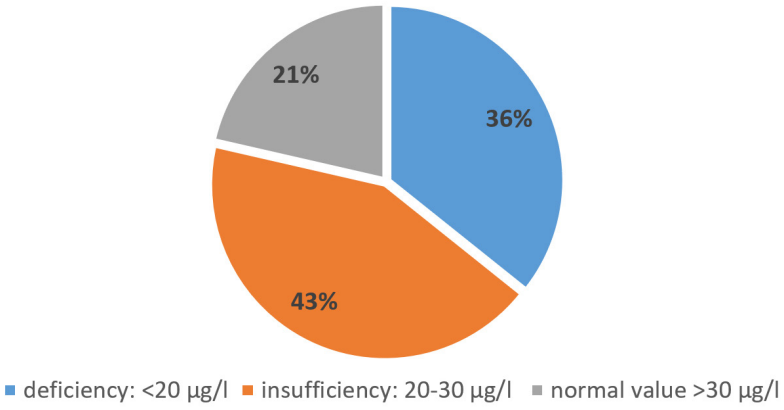

Fig. 1. Serum concentrations of 25-hydroxyvitamin D

Table 1. Baseline characteristics in our two groups

Variables	Vitamin D (N=23)	Placebo (N=18)	p
Age (years)	66.87 ± 9.18	70.5 ± 9.46	0.225
Sex (masculine, %)	43.47	27.7	0.346
Duration of diabetes (years)	15.91 ± 8.85	16.06 ± 10.12	0.963
HbA1C (%)	7.24 ± 0.98	6.79 ± 0.62	0.182
BMI (kg/m^2)	29.46 ± 5.45	30.14 ± 5.31	0.694
GFR (ml/min/1,73 m^2)	72.33 ± 18.7	57 ± 19.93	0.091
Serum 25-hydroxyvitamin D ()	24.71 ± 8.16	14.93 ± 5.85	0.073
Questionnaire score	4.43 ± 1.61	4.17 ± 1.38	0.599
Neuropathic pain (yes, %)	65.2	55.5	0.748
Impaired pain perception (yes, %)	43.47	38.8	0.869
Temperature perception (mode)	2	2	0.763*
Fine touch (mode)	1	0	0.811*
Vibration perception (mode)	1	1	0.179*

*Calculated with Mann Whitney U test

decreased to 43% in the treatment group, but the initial prevalence rate of 56% increased to 78% in the placebo group, $p = 0.003$ (*Figure 2*). Sensory test scores showed an improving trend after cholecalciferol therapy and variable trends in the placebo-control group, but did not reach significance ($p > 0.05$).

We computed the difference between the after and before treatment parameters. In terms of the questionnaire scores, we found that the rate of change was almost three times higher in the vitamin D group compared to the placebo control group. Regarding neuropathic pain, the difference between the two groups was significant, considering that an increase in pain prevalence was observed in the placebo group. For fine touch, there was a moderate positive change in the treatment group, and no improvement in the placebo group. Regarding the temperature- and vibration perception, we observed an opposite change in the two groups with a worsening in the placebo group, however these differences were not statistically significant (*Table 2*).

Discussion

In our study the prevalence of vitamin D deficiency/insufficiency at baseline was very high (79%) compared to some estimates regarding vitamin D deficiency/insufficiency (serum 25(OH) D < 30 nmol/L) prevalence in representative population samples in the US (5.9%), Canada (7.4%) and Europe (13%) [18]. This could be explained by the SARS-COV-2 caused isolation. Several studies regarding the vitamin D effect on pain used different doses and administration methods. The majority of clinical studies involved once-a-week treatment with oral cholecalciferol, with varying weekly doses, 5.000 IU and 40.000 IU for 24 weeks [10], 50.000 IU once weekly for 8 weeks [19], 40.000 IU for 18 weeks [20]. A clinical trial was conducted in which 600.000 IU of vitamin D_3 was administered intramuscularly once, followed by a 20-week follow-up [12]. For our study, the treatment dose of cholecalciferol was set at 2.000 IU per day, so 14.000 IU of cholecalciferol was considered the weekly dose, which was taken for 13 weeks by our patients.

In our study, the subjective symptoms assessed by the questionnaire significantly decreased in the treated group (from 4.43 ± 1.61 to 3.74 ± 1.73, $p = 0.006$), although the difference was marginally significant (0.69 vs. 0.22, $p = 0.094$), due to the low number of cases. In a clinical study in which patients received 50.000 IU of vitamin D_3 once weekly for 8 weeks, a significant reduction in NSS (neuropathy symptom score) was observed in the treated group compared to the control group [19]. Another study investigated the effects of cholecalciferol on neuropathic pain in type 2 diabetic patients, three-quarters of whom were diagnosed with vitamin D deficiency or insufficiency. The group receiving weekly therapy with 40.000 IU over 24 weeks had significantly reduced neuropathy severity score (NSS, NDS-neuropathy disability score, VAS-visual analogue scale) and improved microcirculation and inflammation lesions [10]. The studies mentioned so far have used oral cholecalciferol administration, but in one study patients received a single intramuscular administration of 600.000 IU of vitamin D_3 and significant improvement in positive neuropathy symptoms was observed at 20 weeks follow-up, and also in neuropathic pain (DN4 - Douleur Neuropathique 4, total pain score, SFMPQ - Short Form

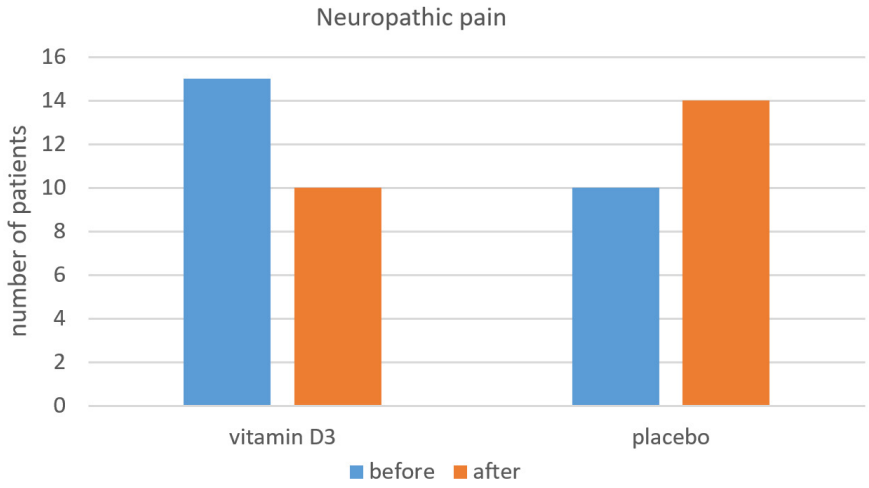

Fig. 2. After-treatment modifications in prevalence of neuropathic pain

Table 2. The rate of change in parameters concerning the two groups

	Vitamin D	Placebo	p
ΔQuestionnaire score	0.69	0.22	0.094
ΔNeuropathic pain	0.21	-0.22	0.003
ΔTemperature perception	0.15	-0.3	0.147*
ΔFine touch	0.1	0	0.650*
ΔVibration perception	0.1	-0.15	0.265*

*calculated with Wilcoxon test

McGill Pain Questionnaire) [12]. These clinical studies suggest that cholecalciferol can have a positive effect on subjective symptoms It seems that a stronger effect can be achieved with increased doses of vitamin D and with extending the duration of therapy.

The neuropathic pain is an important component of the diabetic neuropathic symptoms, having a major impact on the quality of life. The prevalence of the painful form in both our groups initially exceeded 50%, reaching 65.2% in the vitamin D-treated group and 55.5% in the placebo control group. Similar data can be found in the literature. According to Abbott C et al approximately 30-50% of patients with diabetic polyneuropathy experience neuropathic pain, but in more severe forms of neuropathy, this can be as high as 60% [21]. After three months of treatment, significant changes in the prevalence of neuropathic pain were observed, with an initial incidence rate of 65% decreasing to 43% in the cholecalciferol-treated group, while the incidence rate in the placebo control group increased to 78% from 56%. In a meta-analysis, the cholecalciferol-treated group also showed significant improvements in serum 25-(OH)D concentrations, glycated hemoglobin, and McGill Pain Questionnaire scores [22]. In a clinical study, intramuscularly administered cholecalciferol also resulted in significant improvements in positive neuropathy symptoms and neuropathic pain [12]. Further research is needed to clarify the mechanism of action of vitamin D therapy on neuropathic pain, but our study has demonstrated its positive effect on neuropathic pain.

After three months of therapy, no significant change was found in the sensory deficits, and the rate of change in sensory test scores between the two groups also showed no significant difference. These non-invasive tests, in addition to detecting sensory deficits, can also be used to determine whether small- or large fiber involvement is more pronounced. The prick and tip therm tests are suitable for assessing small fiber function, while the vibration and monofilament tests are suitable for assessing large fiber function [1]. In both groups the most affected sensory types were vibration and temperature perception, suggesting that in the majority of patients, neuropathy affects both small and large fibers. Mixed involvement of nerve fibers is the most common form in patients with diabetic polyneuropathy [1], which was confirmed in our study.

The weakness of our study is the low number of patients, which is because a large proportion of patients had received vitamin D prophylaxis before the start of the study.

Conclusions

Vitamin D deficiency/insufficiency is common among diabetic patients, with a prevalence of nearly 80% among our study subjects. Based on the results of our study, we conclude that screening for vitamin D deficiency/insufficiency could be indicated in diabetic patients.

Our results show that in our subjects, oral administration of 2.000 IU of vitamin D_3 (cholecalciferol) for three months significantly reduced subjective symptoms and neuropathic pain. However, there was no significant change in the results of the sensory tests, i.e., no significant improvement in pain perception, temperature awareness, vibration perception, and fine touch awareness.

Author's contributions

LM – conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, visualization, writing – original draft

MIMSZ - conceptualization, data curation, investigation, methodology, project administration, supervision, validation, visualization, writing – review & editing

Conflict of interest

None to declare.

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