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Randomized, Placebo-Controlled, Double-Blind Pilot Study of the Antinociceptive Effect of Menaquinone-7 (Vitamin MK-7) in Patients with Peripheral Neuropathy

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Abstract

A randomized, placebo-controlled double-blind pilot study was conducted to evaluate the efficacy and safety of vitamin MK-7 (menaquinone-7) in comparison to placebo in patients with peripheral neuropathy due to Type 2 diabetes mellitus (DM) or vitamin B12 deficiency (B12D) to facilitate design of a full-size clinical trial. A 100 µg capsule of MK-7 or identical placebo capsule was given twice daily for 8 weeks to 20 ambulatory patients of both genders to evaluate symptoms of peripheral neuropathy in DM and B12D patients. The patients were followed for an additional 4 weeks (total of 12 weeks) and the intensity of the symptoms was self-assessed using a pain Visual Analog Scale (VAS). The serum levels of vitamin MK-7 were determined at the baseline, week 4 and week 8.

There was a statistically significant decrease in the pain VAS score in DM and B12D patients receiving vitamin MK-7 supplementation vs the corresponding placebo groups. The VAS pain score at baseline was on average approximately 9 out of 10. It was reduced to an average score of about 2 by week 12 in the DM and B12D groups supplemented with vitamin MK-7 vs scores greater than 8 by week 12 in the placebo groups. The VAS score was inversely related to MK-7 levels and peripheral neuropathy symptoms. Vitamin MK-7 was well-tolerated without subjective and objective adverse effects reported during the 8-week therapy and during the additional 4 weeks of follow-up.

Keywords: *Vitamin MK-7 Serum Levels; Menaquinone-7 (Vitamin K2-7); Type 2 Diabetes Mellitus; Vitamin B12 Deficiency; Peripheral Neuropathy*

Introduction

Diabetes mellitus peripheral neuropathy (DM) is diagnosed in approximately one-third of patients with diabetes, predominantly Type 2 diabetes mellitus, affecting function and structure of the peripheral nerves in those patients [1-5]. In non-diabetic patients, vitamin B12 deficiency peripheral neuropathy (B12D) is caused by vitamin B12-deficient diets (vegetarian and vegan diets), and as a result of autoim-

mune gastritis, infection with *Helicobacter pylori*, and gastrointestinal disease resulting in dysbacteriosis [6-9]. Vitamin B12 deficiency is highly prevalent in diabetic patients, and an inverse correlation has been shown to exist between diabetic neuropathy and plasma levels of vitamin B12 [10].

Currently, management of DM and B12D is limited to: symptomatic pain relief; body mass index control; glycemic, lipid, and blood pressure control; and vitamin B12 supplementation with monitoring of B12 blood levels [2,11-14]. These treatment modalities are largely ineffective, and peripheral neuropathy continues to be a therapeutic challenge, and one of the causes of musculoskeletal pain, morbidity, and disability [14-16].

A recent clinical observation has indicated that vitamin MK-7, also known as vitamin K2-7, at a dose of 100 μg twice a day for several weeks may be safe and effective in the management of peripheral neuropathy [17,18]. Vitamin K occurs in nature as phylloquinone (vitamin K1) and menaquinone (vitamin K2), with its primary function as a calcium chaperone, controlling a family of at least 18 calcium and vitamin K-dependent proteins, sometimes referred to as a multitasking vitamin [19].

The present study was a 12-week randomized, placebo-controlled, double-blind pilot trial employing vitamin K2 as the long-chain menaquinone-7 (or MK-7), in managing peripheral neuropathy in patients with clinically diagnosed Type 2 diabetes mellitus (DM) or patients presenting with vitamin B12 deficiency (B12D).

Materials and Methods

Study design

The randomized, double-blind, placebo-controlled, 12-week parallel small sample (pilot) study was conducted to compare tolerability and efficacy of long chain menaquinone-7 (MK-7) in patients with Type 2 diabetes mellitus (DM) or vitamin B12 deficiency (B12D) presenting with peripheral neuropathy. The 4-month study was approved by the Independent Ethics Committee, Mumbai, India dated March 18, 2019. The trial was conducted at Kokan Hospital, Mumbai, India. The study was registered with Clinical Trials Registry-India (CTRI) registered on 06/06/2019 as CTRI/2019/06/019548. CTRI is searchable from the World Health Organization's'(WHO's) International Clinical Trials Registry Platform (ICTRP) (http://ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=32668&EncHid=&userName=synergia%20life) as well as from CTRI (www.ctri.nic.in). The study was conducted in accordance with the International Conference on Harmonization of Good Clinical Practice (ICH-GCP E6)(R2) Consensus Guidelines, ethical principles based on the Declaration of Helsinki, and the applicable regulatory requirements.

Subjects

Twenty-five patients with clinically confirmed Type 2 diabetes mellitus (DM) or vitamin B12 deficiency (B12D) and peripheral neuropathy were considered for the study. A detailed flow chart of the various steps taken in the study from initial patient assessment through data analysis is provided in figure 1.

Neuropathy was diagnosed by the principal investigator (PI), a physician, in patients with a history of diabetes or history of standalone persistent low serum levels of vitamin B12. Neuropathy was diagnosed based on the presence of symptoms of peripheral neuropathy including decreased sensation, positive neuropathic sensory symptoms e.g., "asleep numbness," prickling or stabbing sensation, burning or aching pain predominantly in the toes, feet, or legs, accompanied by symmetric decrease of distal sensation or decreased/absent ankle reflexes. On physical examination, a symmetrical stocking like distribution of sensory abnormalities more commonly in both lower limbs and in more severe cases in upper limbs were noted.

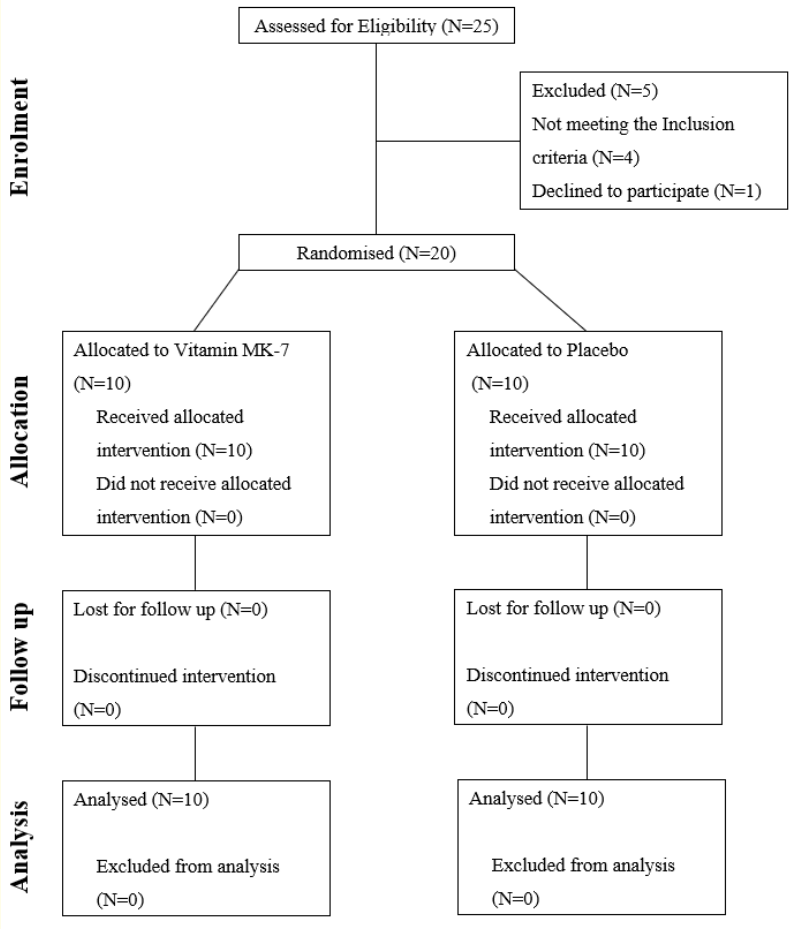


Figure 1: *Flow chart of MK-7 in peripheral neuropathy study.*

Inclusion criteria:

1. Male and female subjects 18 to 65 years old suffering from DM or B12D.
2. Symptomatic neuropathy score > 4 on the Visual Analogue Scale (VAS).
3. Willing to sign the informed consent.

Exclusion criteria:

1. Suffering from systemic illness other than Type 2 diabetes mellitus or vitamin B12 deficiency.
2. Patients who are on corticosteroids.
3. Patients who are on coumarin analogues.

4. Patients who are on quinine hydrochloride.

5. Patients on oral contraceptives.

6. Pregnant or nursing mothers.

7. Participation in clinical trials evaluating investigational pharmaceuticals or biologics within 3 months, or devices within 30 days of admission to the study.

8. History of alcohol and/or substance abuse within one year prior to admission to the study.

Twenty-five patients with an initial diagnosis of Type 2 diabetes mellitus (DM) and/or vitamin B12 deficiency (B12D) suffering from peripheral neuropathy, were seen by the principal investigator (PI) in the Department of Medicine outpatient clinic, Kokan Hospital, Mumbai, India. Upon anamnesis and physical examination, the diagnosis was confirmed and the eligibility criteria based on the Protocol approved by the Ethics Committee was reviewed with each patient. Of the 25 subjects, 4 patients did not meet the admission criteria and one subject declined participation in the study. The informed consent approved by the Ethics Committee was reviewed with the individual patients, explaining the printed image of Visual Analog Scale (VAS). Each subject was asked to point out the number from 0 to 10 (0 - no pain, 10- unbearable pain), based on the severity of his/her symptoms of peripheral neuropathy. Based on a severity score above 4, 20 patients (11 men and 9 women), were eligible for the study and the informed consent was signed by each individual patient and the PI (Figure 1).

The patients with diabetes mellitus (DM), 5 women and 4 men, had a disease history ranging from 5 months to 7 years with peripheral neuropathy lasting from 1 month to 8 months. The patients with vitamin B12 deficiency (B12D), 4 women and 7 men, had a history of vitamin B12 deficiency ranging from 3 months to 9 months and peripheral neuropathy duration for 1 month to 7 months. The age of the diabetic patients was 38 to 40 years old while those of vitamin B12 deficiency patients was 47 to 49 years old. The 9 patients with DM continued their preadmission regimens throughout the 12-week study with 500 mg three times daily of the oral antidiabetic drug Metformin (class of biguanides), combined in some patients with the dipeptidyl peptidase-4 (DPP-4) Teneligliptin 20 mg taken three times daily. The 11 patients with B12D received supplements with vitamin B complex (Neurobion Plus, Neurobion Forte, Cobadex-Z and Becosules-Z) including vitamin B12 in a dose ranging from 15 - 750 µg/day.

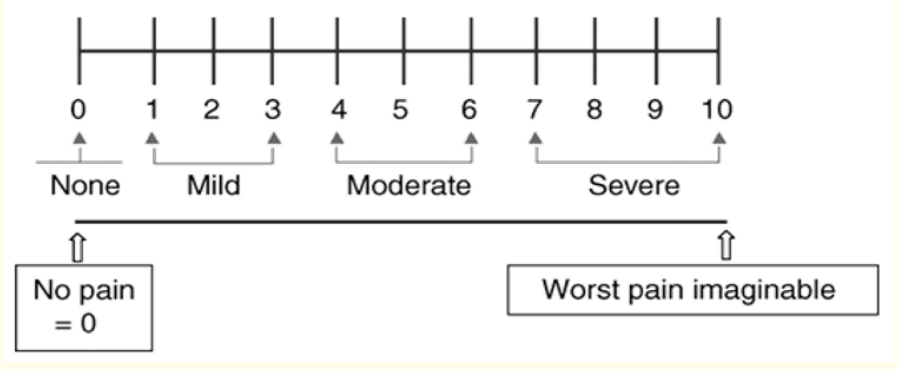
The study procedure

The test substance (vitamin MK-7 100 µg per hard-gel capsule) as well as the identical placebo capsules were manufactured and provided by Synergia Life Sciences Ltd. Pvt., Mumbai India. Each product was packed 30 capsules per bottle and prepared by the pharmacist in Synergia Life Sciences pharmacy. The pharmacist coded each bottle containing vitamin MK-7 or placebo and was therefore the only person in the research team who knew the contents of the bottles.

The patients were randomized by the PI at the outpatient clinic, Kokan Hospital, with a simple numerical randomization method and assigned a Subject Identification within the DM and B12D groups. The randomized groups of patients received the coded bottles with study substance and were instructed to take daily one capsule with a breakfast and one capsule with a dinner for 8 weeks.

The patients were examined by the PI at baseline, week 2, week 4, week 6, week 8, and week 12. The subjective severity of the symptoms of peripheral neuropathy was assessed with a validated pain VAS scale, as described in the literature and summarized in graphic form below ---- from 0 to 10 score (0 - no pain, 10 - unbearable pain) at baseline and thereafter every second week until week 12 [20].

Visual analog scale (VAS) with gradation of the peripheral neuropathy



The gradation of peripheral neuropathy pain based on the following criteria:

0 - 10 points visual analog scale (VAS):

0 = Absent; 1 - 3 = Mild; 4 - 6 = Moderate; 7 - 10 = Severe.

Subjective and objective side effects and adverse events were assessed at all study time points. Therapeutic activity was measured by assessing the reduction in the severity of the symptoms of peripheral neuropathy, including: pain, tingling, pricking sensation, burning sensation, and cramps along with a physical weakness and fatigue. These signs and symptoms were compared to baseline and the placebo-receiving study group at the end of the study.

Once the 12-week study was completed, the codes of vitamin MK-7 and placebo receiving subjects were decoded by the pharmacist and handed over to the investigators for evaluation and statistical analysis of the results.

Determination of vitamin K (MK-7) serum levels

Blood samples from the patients for vitamin MK-7 analysis were obtained at baseline, week 4 and week 8. The blood samples were allowed to clot for one hour at room temperature. The samples were then centrifuged for 10 minutes at approximately 1000g and stored at 2 - 8°C. Each serum sample was transferred immediately into a clean pre-labeled polypropylene tube using a Pasteur pipette tube in singlet and stored upright in a freezer at a temperature -65 ± 10°C for interim storage as required.

The serum samples were transferred to Synergia Life Sciences Ltd. Pvt. laboratory under cold conditions (-65 ± 10°C) for analysis of vitamin MK-7. A data logger was provided to ensure the storage temperature conditions. The analysis of the samples was performed using a calibration curve with quality control samples. Serum samples were assayed for vitamin MK-7 using a single HPLC method with fluorescence detection, which was validated according to the international guidelines [21,22].

A reverse phase HPLC method [1-3] involving post column derivatization and fluorescent detection was used with some modifications. Analytical standards for vitamins MK-7, the internal standard K2-6 and zinc dust (< 10 µM) for post column reduction of vitamin MK-7 were purchased from Sigma Chemical Co. All the other chemicals used were Analytical Reagent grade and all solvents for HPLC were HPLC grade.

Briefly, a HPLC (Shimadzu) was used that was equipped with degasser DGU_20A5R, pump LC-20AD, auto-sampler SIL-20AC HT, column oven CTO-10 AS and fluorescence detector RF 20A. A reverse phase C18 column (Kinetex C18, 10 cm x 4.6 mm, 2.6 µ, 100°A) was used with column oven adjusted to 25°C. A stainless-steel post column (30 x 4 mm) assembly was placed between the analytical column, which was packed with zinc dust for the post column reduction of vitamin K, and the detector. The mobile phase solution was prepared as follows: 2 ml of zinc solution (0.136% zinc chloride, 0.04% sodium acetate, 30 µL acetic acid in 75% methanol) in 2 liters of methanol and filtered through 0.45 µm filter paper. Injected samples or standards (50 µl) were eluted at 1.2 ml/min isocratic flow rate and a run time of 12 minutes. The chromatogram was monitored using a fluorescent detector at excitation and emission wavelengths 248 nm and 430 nm, respectively.

The analytical method was established and validated as per the ICH guidelines [21,22]. A calibration curve for vitamin MK-7 was established in the range of 0.15 to 20 ng/ml. The limit of detection (LOD) for vitamin MK-7 was 0.08 ng/ml, and the limit of quantitation (LOQ) was 0.15 ng/ml with signal to noise ratio of 5:1.

Synergia Life Sciences laboratory participates with UK Vitamin K External Quality Assurance Scheme (KEQAS) for quality control. On six occasions, two serum and one standard vitamin K sample were received from KEQAS for the estimation of vitamin K by the HPLC method. The target for results was ± 20% deviation from the ALTM (all laboratory trimmed mean representing the target concentration). In all six occasions the method developed gave 'Satisfactory' results with a 'Green' certification.

Determination of vitamin B12

Venous blood samples were collected on an empty stomach. The samples were centrifuged within one hour of collection and the sera were stored at -40°C until analyzed. Serum vitamin B12 was measured by the chemiluminescence, competitive immunoassay method using a commercial automatic electrochemical immuno-analyzer (Roche E170) and electrochemiluminescence immunoassay (ECLIA) kit (Immulite 2000-BIODPC).

Organ function monitoring

Blood chemical analyses included blood morphology, erythrocyte sedimentation rate (ESR), vitamin B12, homocysteine, glycosylated hemoglobin, fasting and post-prandial plasma glucose, prothrombin time-international normalized ratio (PT-INR), liver function tests, and renal function tests at baseline, week 4, week 8 and week 12. All routine blood parameters were performed at the National Accreditation Board for Testing and Calibration Laboratories (NABL) Accredited Laboratory of the study site, Kokan Hospital in Mumbai, India.

Complete blood counts were determined using a PC 210 ERMA Blood Cell Counter. ESR was determined by the Wintrobe method. Liver function test and renal function test were assessed by standard biochemical blood chemistry methods. Prothrombin time was done by the coagulation method. Fasting and post-prandial plasma glucose was estimated by the glucose oxidase-peroxidase (GOD-POD) enzymatic method. Glycosylated hemoglobin was measured by the boronate affinity test with a Nycocard device. Vitamin B12 and homocysteine were determined by RIA/ELISA/CLIA methods.

Intervention drug, dosage, and compliance

Vitamin MK-7 (100 µg) and the identical placebo were manufactured by Synergia Life Sciences Pvt. Ltd., in the form of capsules packaged 30 capsules per bottle. The capsules were supplied to patients at the time of the enrollment and every 15 days thereafter during the course of the study. Patients ingested 100 µg MK-7 capsule or the placebo twice a day, every morning and evening after eating, for 8 weeks. The supplement compliance was assessed by counting the capsules in the bottle brought back at the follow-up visits.

Statistical analysis

Statistical analysis and significance involving groups with small sample size were performed as described by de Winter [23]. Data of the 20 patients were analyzed by using the unpaired *t*-test method. This method was used to determine the significance of two sets of data. Analyses were made between various time points within a treatment group and between placebo and treated at each time point. Coefficient correlations between levels of vitamin MK-7 and vitamin B12 were determined by using Trend Analysis. A Microsoft Excel program was used to plot linear fit of vitamin B12 levels vs. vitamin MK-7 levels. Trend line, linear equation and R^2 values were determined using a Microsoft Excel program. The pilot cohort does not have statistical power, but the study helps establish the necessary components for a larger randomized, placebo-controlled, double-blind study to be conducted in the future.

Results

Of the 9 patients with Type 2 diabetes mellitus (DM) and 11 patients with vitamin B12 deficiency (B12D) and peripheral neuropathy allocated to the study, all subjects completed the allocated intervention, none discontinued intervention, none was lost to follow-up, and none was excluded from the analysis of the results (Figure 1 and table 1).

	Type 2 diabetes mellitus group				Vitamin B12 deficiency group			
	Vitamin MK-7		Placebo		Vitamin MK-7		Placebo	
	Male	Female	Male	Female	Male	Female	Male	Female
N	2	2	2	3	4	2	3	2
Age (yrs.)	35.00 ± 4.24	35.50 ± 0.71	47.00 ± 12.73	40.00 ± 9.54	45.50 ± 8.35	49.00 ± 8.49	54.00 ± 7.81	44.00 ± 2.83

Table 1: *Demographics of the patients in each of the experimental groups.*
Values expressed as Mean ± SD; SD- Standard Deviation.

The average baseline VAS score for DM and B12D patients was approximately 9, indicating a severe, self-assessed pain including tingling, prickling and burning sensation, combined with muscular weakness, and numbness accompanied by general fatigue. The B12 group also experienced frequent muscle cramps (Table 2 and 3).

By the end of week 4 of the study, the VAS score in DM and B12D patients receiving vitamin MK-7 supplement had improved to approximately 4 with a reduction in the intensity of the pain symptoms as compared to the placebo groups. Patients receiving MK-7 for 4 weeks reported better quality of daily life with reduced feeling of weakness and fatigue and less burning pain in DM and less intensity of muscle cramps in B12D patients (Table 2 and 3).

	Tingling		Pricking sensation		Cramps	
	Vit. MK-7	Placebo	Vit. MK-7	Placebo	Vit. MK-7	Placebo
No. of subjects	6	5	6	5	6	5
Baseline	8.5 ± 0.5^{a}	8.8 ± 0.8^{a}	8.7 ± 0.8^{a}	8.8 ± 0.8^{a}	8.3 ± 0.5^{a}	8.4 ± 0.5^{a}
End of week 2	5.7 ± 1.0^{b}	8.4 ± 0.5^{a}	6.0 ± 1.4^{b}	8.4 ± 0.5^{a}	7.2 ± 1.5^{ab}	7.6 ± 0.5^{a}
End of week 4	3.7 ± 0.8^{c}	8.2 ± 1.1^{a}	3.7 ± 0.8^{c}	8.2 ± 1.1^{a}	5.2 ± 1.3^{b}	8.2 ± 0.8^{a}
End of week 8	1.5 ± 0.5^{d}	8.4 ± 0.5^{a}	1.5 ± 0.5^{d}	8.4 ± 0.5^{a}	2.3 ± 0.5^{c}	8.4 ± 0.5^{a}
End of week 12	2.2 ± 0.4^{d}	8.8 ± 0.8^{a}	2.2 ± 0.4^{d}	8.8 ± 0.8^{a}	1.5 ± 0.5^{c}	8.2 ± 0.4^{a}

Table 2: *VAS scores in type 2 diabetes mellitus patients receiving vitamin K (MK-7) or placebo.*
Values are expressed as mean ± SD (Standard Deviation). Values within columns as well as with respect to the corresponding placebo group with non-identical superscript letters are statistically significant ($p < 0.05$).

By the end of week 8, the VAS scores of patients with DM and B12D supplementing with MK-7 had improved to approximately 2. The tingling, prickling sensation, and numbness had reduced along with a decrease in the muscular weakness and feeling of fatigue. Cramps and burning pain were occasional and decreased in intensity (Table 2 and 3).

By the end of week 12, 4 weeks after discontinuing MK-7 and placebo supplements, the VAS score of patients with DM and B12D previously on the vitamin supplement held at an average of about 2. The VAS score from baseline to 12 weeks in the DM and B12D patients receiving placebo consistently ranged from 8 to 9 at all testing points. Patients receiving placebo were not feeling better with a persistent weakness and fatigue, and unrelenting intensity of cramps in vitamin B12 deficient patients and burning pain sensation in DM patients (Table 2 and 3).

	Tingling		Pricking sensation		Burning	
	Vit. MK-7	Placebo	Vit. MK-7	Placebo	Vit. MK-7	Placebo
No. of subjects	4	5	4	5	4	5
Baseline	8.8 ± 0.5[a]	8.2 ± 0.4[a]	8.8 ± 0.5[a]	8.2 ± 0.4[a]	8.8 ± 0.5a[a]	8.4 ± 0.5[a]
End of week 2	5.3 ± 1.0[b]	8.2 ± 0.4[a]	5.3 ± 1.0[b]	8.2 ± 0.4[a]	7.8 ± 1.0[a]	7.6 ± 0.5[a]
End of week 4	3.0 ± 0.8[c]	8.2 ± 0.8[a]	3.0 ± 0.8[c]	8.2 ± 0.8[a]	5.5 ± 0.6[b]	7.8 ± 0.4[a]
End of week 8	1.5 ± 0.6[d]	8.6 ± 0.5[a]	1.5 ± 0.6[d]	8.6 ± 0.5[a]	2.0 ± 0.0[c]	7.8 ± 0.4[a]
End of week 12	2.0 ± 0.0[d]	8.0 ± 1.2[a]	2.0 ± 0.0[d]	8.0 ± 1.2[a]	1.5 ± 0.6[c]	8.4 ± 0.9[a]

Table 3: *VAS scores in vitamin B12 deficiency patients receiving vitamin K (MK-7) or placebo.*

Values are expressed as mean ± SD (Standard Deviation). Values within columns as well as with respect to the corresponding placebo group with non-identical superscript letters are statistically significant ($p < 0.05$).

The baseline values of vitamin MK-7 in serum in subjects in all groups ranged from about 0.4 - 0.6 ng/ml (Table 4). The week 4 and week 8 levels of vitamin MK-7 in serum in all subjects (DM and B12D) receiving vitamin MK-7 were approximately 14 ng/ml and 10 ng/ml, respectively, and in the placebo groups were approximately 0.8 ng/ml and 0.40 ng/ml, respectively (Table 4), reflecting the daily intake of MK-7 vs placebo.

	Type 2 Diabetes mellitus		Vitamin B12 Deficiency	
	Vitamin MK-7	Placebo	Vitamin MK-7	Placebo
No. of subjects (N)	4	5	6	5
Baseline	0.5 ± 0.1[a]	0.6 ± 0.2[a]	0.4 ± 0.3[a]	0.4 ± 0.2[a]
End of week 4	13.6 ± 6.7[b]	0.8 ± 0.2[a]	9.4 ± 6.5[b]	0.4 ± 0.1[a]
End of week 8	13.9 ± 8.9[b]	0.8 ± 0.5[a]	10.0 ± 7.1[b]	0.5 ± 0.3[a]

Table 4: *Vitamin MK-7 levels from baseline to end of Week 8 MK-7 supplementation.*

Values are expressed as mean ± SD (Standard Deviation). Values within columns as well as with respect to the corresponding placebo group with non-identical superscript letters are statistically significant ($p < 0.05$).

Blood chemistries and hematology as well as organ function tests were performed at baseline, week 4, week 8, and week 12. Glycosylated hemoglobin levels (normal 4 - 6%) in patients with DM receiving placebo and vitamin MK-7 were elevated throughout the study with no effects of vitamin MK-7 supplementation. In patients with B12D, glycosylated hemoglobin levels remained within the normal range

from baseline to week 12. Fasting (normal 60 - 100 pg/dL) and postprandial (> 160 pg/dL) glucose in DM patients was elevated throughout the study with no effects of vitamin MK-7 supplementation. Blood glucose levels in the B12D group remained within the normal range throughout the study (data not shown).

The vitamin B12 levels (normal 245 - 900 pg/mL) were within the normal range at baseline and throughout the 12-week study in DM placebo and MK-7 supplemented patients, and below the normal values in B12D patients receiving placebo or vitamin MK-7 (Table 5). However, the DM and B12D patients receiving vitamin MK-7 showed small, incremental increases in circulating vitamin B12 levels from baseline to weeks 4, 8, and 12, although the increases were not statistically significant. By comparison, vitamin B12 levels in the corresponding patient groups receiving placebo remained unchanged from baseline to week 12 (Table 5).

The coefficient factors (R^2) for serum levels of vitamin B12 in the B12 deficient group supplemented with vitamin K2-7 decreased during the course of the study, i.e. at baseline (R^2 = 0.2612), 4th week (R^2 = 0.0008) and 8th week (R^2 = 0.0005). The coefficient factor for serum levels of vitamin B12 in the diabetic group supplemented with vitamin K2-7 also decreased, i.e. at baseline (R^2 = 0.087), 4th week (R^2 = 0.0224) and 8th week (R^2 = 0.0045). The coefficient factors for serum levels of vitamin B12 receiving placebo were unchanged in the course of the study in B12 deficient and diabetic groups. These statistical data indicate that vitamin K2-7 treated groups had improved vitamin B12 serum levels compared to placebo receiving groups.

Type 2 diabetes mellitus patients-vitamin B12 levels (pg/mL)*				
Weeks	**Male**		**Female**	
	Vitamin MK-7 therapy	**Placebo**	**Vitamin MK-7 therapy**	**Placebo**
0	288.5 ± 0.7	337.0 ± 1.4	313.5 ± 53.0	287.0 ± 49.6
4	293.0 ± 7.1	338.0 ± 2.8	323.5 ± 48.8	287.3 ± 49.1
8	296.0 ± 14.1	338.0 ± 2.8	327.5 ± 48.8	287.3 ± 49.1
12	296.5 ± 17.7	339.0 ± 1.4	335.5 ± 55.9	289.3 ± 50.0
Vitamin B12 deficiency patients-vitamin B12 levels (pg/mL)				
Weeks	**Male**		**Female**	
	Vitamin MK-7 therapy	**Placebo**	**Vitamin MK-7 therapy**	**Placebo**
0	168.3 ± 21.2	165.3 ± 35.9	160.0 ± 2.8	156.0 ± 17.0
4	169.5 ± 20.7	165.3 ± 36.7	161.5 ± 4.9	158.0 ±1 7.0
8	171.5 ± 21.3	168.0 ± 36.4	164.0 ± 5.7	158.0 ± 17.0
12	173.3 ± 19.9	168.7 ± 37.0	165.0 ± 4.2	158.0 ± 19.8

Table 5: *Vitamin B12 (normal range 245-900 pg/mL) levels for the male and female patients with peripheral neuropathy due to type 2 diabetes mellitus or vitamin B12 deficiency receiving vitamin MK-7 or placebo.*

Values expressed in Mean ± SD; SD- Standard Deviation.

**: Coefficient correlation (R^2) decreased with vitamin MK-7 supplementation in DM patients.*

The mean corpuscular volume (MCV) values in patients with B12 deficiency and peripheral neuropathy were within upper normal limits throughout the 12-week study and not affected by vitamin MK-7 supplementation. The MCV values in DM patients also remained

within normal limits throughout the study (data not shown). Based on MCV values and normal hematological parameters, the B12D and DM patients did not present with megaloblastic anemia at the time of accruement to the study and during its duration.

The additional blood studies including blood morphology, ESR, homocysteine, PT-INR, liver function tests, and renal function tests were conducted at baseline, week 4, and week 12, and were within normal limits throughout the study in all study patients (data not shown).

Tolerability and safety

Vitamin MK-7 capsules were well tolerated by all patients during the 8 weeks of administration. No side effects or subjective and objective adverse events were reported during the period of therapy and follow-up in all 20 patients.

Compliance

Monitoring of the capsule intake during the 8 weeks of the trial indicated that the intake was regular by the patients. Capsule consumption was monitored by counting the number of capsules remaining in the bottle at the end of 15 days from the date of dispensing the bottle in each phase of the study.

Discussion and Conclusion

This pilot randomized, placebo-controlled double-blind study provides, for the first time, evidence linking the increased levels of circulating vitamin MK-7 to improvement in the debilitating pain, cramps, and symptoms of peripheral neuropathy in diabetes Type 2 and vitamin B12 deficiency---two distinct clinical conditions linked by symptomatology and possibly underlying pathology [10].

The intervention with the long-chain vitamin MK-7 in the peripheral neuropathy patients may involve vitamin K in its calcium chaperone multitasking role, carboxylating and activating a family of proteins with the glutamic acid residues which maintains the cardiovascular, immune, metabolic, neuro-muscular, and bone-building functions [19].

One of the emerging possibilities is the peripheral neuropathy as an inflammatory autoimmune condition that damages the myelin sheet of peripheral nerves with vitamin K operating through the carboxylation of growth arrest-specific gene 6 (GAS6) protein to stimulate oligodendrocytes and myelin sheet repair [24-27].

Another vitamin K-dependent protein, matrix Gla protein (MGP), may alleviate neuropathy by improving cardiovascular and endothelial functions and oxygenation of peripheral nerves [19,28,29]. The high-circulating concentrations of uncarboxylated and inactive MGP (ucMGP) have been shown to correlate positively with cardiovascular and endothelial dysfunction, and supplemental MK-7 has been shown to reduce ucMGP levels in a cohort of mixed gender adults [29].

Vitamin MK-7 may also improve peripheral neuropathy, preventing Type 2 diabetes and alleviating its clinical course by carboxylation and activation of osteocalcin, a hormone which increases insulin sensitivity in humans [30,31].

Based on the current study, supplemental vitamin MK-7 in the DM and B12D groups may help increase and/or maintain circulating vitamin B12, enhancing its biological role in prevention of peripheral neuropathy in patients with diabetes and the B12 vitamin deficiency.

In this pilot 12-week study, the circulating levels of vitamin MK-7 increased significantly following 2, 4 and 8 weeks of supplementation, along with a statistically significant decrease vs. baseline (p value < 0.05) in VAS scores. Patients in both study groups, with diabetes and B12 deficiency, showed good tolerability of vitamin MK-7 supplementation with no subjective or objective side effects of the therapy.

The trend analysis showed that there was correlation between vitamin MK-7 supplementation and serum levels in both DM and B12D patients, with a greater effect in DM patients as compared to B12D subjects.

Finally, this study focused on the effects of MK-7 on neuropathic pain in these patients. It should be noted that the strengths of this pilot study were the antinociceptive effects of vitamin MK-7 in subjects with neuropathic pain associated with diabetes and vitamin B-12 deficiency. The *t*-test was used to compare pairs of mean values. The *t*-test when used with extremely small sample sizes has been shown to provide Type I error rates close to the 5% nominal value ($p < 0.05$) [23]. In summary, the results indicate a significant potential benefit of MK-7 for the management of neuropathic pain and provides a frame-work for conducting a larger randomized control trial. This study is a sequel of the study published in EC Neurology on August 1, 2021 [32].

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Disclosures

DSM and SSJ are in the management of Synergia Life Sciences. MD is the Principal Investigator. RV and ADBV are medical consultants. VB serves on an advisory board of Synergia Life Sciences Pvt. Ltd. SJS has no potential conflicts to disclose.

Data Sharing

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