Research Article

Role of Vitamin B12 supplementation on incipient neuropathy in patients with type II diabetes mellitus

Puvvada Ranadheer Chowdary[1], D. Praveen[1], M. Vijey Aanandhi[2]*

ABSTRACT

Diabetic peripheral neuropathy (DPN) affects approximately 44% of older diabetics. Some DPN patients may experience extremely painful symptoms, whereas those who have a more marked neuropathic deficit may not have any symptoms. Deficiency of Vitamin B12 (also known as cobalamin), which results in a lack of methylcobalamin, has been associated with significant neurological pathology, especially peripheral neuropathy. It is also associated with the onset of diabetic neuropathy. In patients with DPN, Vitamin B12 deficiency may be caused by the use of antidiabetic agents such as metformin. This was a randomized controlled trial with a parallel assignment of two arms. All two parallel trial arms will be enrolled as per per-protocol analysis. The interventional model included Metformin 500 mg BD+pregabalin 75 mg+cyanocobalamin 100 mg OD. Every patient will be followed up for 9 months. Serum Vitamin B12 levels will also be assessed at the same time intervals. The trial arm showed significant improvement ($P < 0.05$*) in the pain control, serum homocysteine, and methylmalonic acid statuses compared to the placebo arm receiving pregabalin. Vitamin supplementation proves to be efficient in management of DPN.

KEY WORDS: Diabetes mellitus, Diabetic neuropathy, Vitamin B12

INTRODUCTION

Diabetic peripheral neuropathy (DPN) affects approximately 44% of older diabetics. Some DPN patients may experience extremely painful symptoms, whereas those who have a more marked neuropathic deficit may not have any symptoms.[1-4] Chronic sensorimotor neuropathy is characterized by pain, paraesthesia, and sensory loss. Several pathogenic mechanisms contribute to the DPN etiology, including microangiopathy and oxidative stress. Current clinical management guidelines for DPN are limited to adequate glucose control and symptomatic pain relief.[5-7]

Deficiency of Vitamin B12 (also known as cobalamin), which results in a lack of methylcobalamin, has been associated with significant neurological pathology, especially peripheral neuropathy. It is also associated with the onset of diabetic neuropathy.

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

In patients with DPN, Vitamin B12 deficiency may be caused by the use of antidiabetic agents such as metformin.[8-10]

For many years, Vitamin B12 and its coenzymes have been used to treat pain. In some countries, Vitamin B12 is categorized as an analgesic drug. It has been suggested that Vitamin B12 may increase the availability and effectiveness of noradrenaline and 5-hydroxytryptamine in the descending inhibitory nociceptive system. In animal models, morphological and histological evidence has also shown that long-term administration of methylcobalamin promotes the synthesis and regeneration of myelin.[11-13]

The aim of the study is to study the role of Vitamin B supplementation in patients with type II diabetes mellitus on incipient neuropathy.

METHODOLOGY

Sample Size

The sample size is calculated using Raosoft online sample size calculator. For a random sampling of a population of 1000 persons with a confidence interval

[1]Research Scholar, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Chennai, Tamil Nadu, India, [2]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. M. Vijey Aanandhi, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Chennai, Tamil Nadu, India. E-mail: hodpchemistry@velsuniv.ac.in

Received on: 17-05-2019; Revised on: 20-07-2019; Accepted on: 24-08-2019

of 95%, the sample size is found to be 278 with a 5.75% margin of error.

$S.S=Z^2 P (1-P)/C^2$
Z=1.96, C.I=95%, P=0.5
S.S=384
New S.S= S.S/1+(S.S−1)/Pop
S.S=384, Pop=100
New S.S=79

Hence, 80 patients will be included to overcome subject withdrawal from the study.

Study Design

This was a randomized controlled trial with a parallel assignment of three arms. All three parallel trial arms will be enrolled as per per-protocol analysis.

This is a single-blinded study with randomized permuted blocks where parallel assignment of three arms is done. It is a safety/efficacy analysis.

Table 1: Baseline characteristics of included patients

Characteristics	Group A	Group B
Age in years (mean±SEM)	49.6±3.8	51.4±3.2
Male (*n*=51)	27	24
BMI (mean±SEM)	24.2±2.4	23.6±2.8
Family history (DM)	15	12
Fasting blood sugar mg/dl	198.6±13.2	204.2±3.8
Post-prandial blood sugar mg/dl	262±7.2	279±13.6
Glycated hemoglobin%	8.1±1.0	8.4±0.8
Serum homocysteine	33.1±2.7	32.6±2.1
C-reactive protein	2.5±0.2	2.9±0.1
Methylmalonic acid	3.2±3.1	4.7±2.6

All values are mean±SEM unless otherwise mentioned. There is no significant difference between both the groups in the baseline parameters

Table 2: HbA1C levels

HbA1C levels	0 month	3rd month	6th month	9th month
Group A	8.1±1	7.9±0.6	7.1±0.2	6.9±0.1
Group B	8.4±0.8	7.8±0.4	6.9±0.2	6.6±0.3

All values are mean ± SEM. It is observed that on performing Analysis of Variance $P = 0.0512$ which is not significantly different. HbA1C: Glycated hemoglobin

The parameters such as glycated hemoglobin (HbA1C), fasting blood sugar, post-prandial blood sugar, lipid profile, renal function tests, cardiac function tests, serum homocysteine, methylmalonic acid (MMA), and C-reactive protein levels will be assessed on 4 time intervals such as (before the initiation of intervention, 3rd month, 6th month, and 9th month).

Methylmalonic acid (MMA) is a compound that reacts with Vitamin B-12 to produce coenzyme A (CoA). CoA is essential to normal cellular function. When Vitamin B-12 deficiencies occur, MMA levels increase.

Every patient will be followed up for 9 months. Serum Vitamin B levels will also be assessed at the same time intervals.

The study protocol was submitted for the Institutional Ethical Committee Screening, and their approval was obtained VISTAS-SPS/IEC/2018/I/01.

Inclusion Criteria

All the patients above 18 years of age who are diagnosed with type II diabetes mellitus (T2DM) and taking metformin alone were included in the study. Patients having fasting blood glucose levels in the range of 126-250mg/dL and HbA1C in the range of 7.0–9.9% (since higher levels may require multiple drugs) are included in this study. Patients with established DPN and are only on pregabalin are also included in this study.

Exclusion Criteria

Individuals unable to give informed consent, patients who are diagnosed with tuberculosis, patients who are diagnosed with peripheral vascular disease, patients with injury to their legs, those who had taken antibiotics in the last month, those with a medical history of significant gastrointestinal disease, for example, inflammatory bowel disease, those who had undergone a previous bowel resection, and individuals taking diabetes medication other than metformin. Routine investigations such as electrocardiogram, serum electrolytes, blood urea, and creatinine, and liver

Table 3: Biomarker evaluation

Parameters	0 month	3th month	6th month	9th month
C-reactive protein levels				
Group A	2.5±0.2	2.8±0.3	2.3±0.2	2.1±0.6
Group B	2.9±0.1	2.2±0.4	1.9±0.2	1.4±0.3*
Serum homocysteine levels				
Group A	33.1±2.7	30.4±2.6	25.3±2.6	24.1±0.9
Group B	32.6±2.1	29.2±2.1	23.2±2.6	19.1±0.5*
Methylmalonic Acid Levels				
Group A	3.2±3.1	3.4±2.9	3.4±2.7	3.1±2.9
Group B	4.7±2.6	3.3±2.16	2.4±2.14*	1.9±2.1**

All values are mean±SEM.*P<0.05 which is significantly different. **P<0.001 which is extremely significant

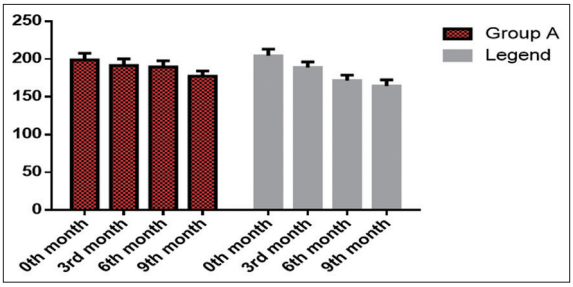

Figure 1: Fasting blood sugar levels (mg/dL). All values are mean±SEM. It is observed that on performing Analysis of Variance $P = 0.0042$* which is significantly different

function tests were performed to exclude active medical problems in all patients. Patients who are using Opiod analgesics are excluded.

Statistical Analysis

Standard descriptive statistics, including means, standard deviations, frequencies, and percentages as appropriate will be used to summarize the demographic, anthropometric, and laboratory results across participants grouped by fasting glucose and T2DM treatment. These variables are described using geometric means and 95% confidence intervals. Associations between the clinical characteristics of the cohorts grouped by fasting glucose (including those treated with Metformin) were tested using one-way analysis of variance (ANOVA) and Chi-squared tests as appropriate. The univariate associations between plasma Vitamin B12 and demographic, anthropometric, and laboratory measures were tested using Pearson's Correlation coefficients and one-way ANOVA. Significant predictors identified from these univariate analyses were then combined in a multiple regression analysis to identify significant independent associations with plasma Vitamin B. The two-tailed $P < 0.05$ was taken to indicate statistical significance. Efficacy analysis will be performed using Chi-squared test. All statistical analyses were undertaken using SPSS.

RESULTS

The demographics, anthropometry and baseline data is expressed in Table 1. It could be seen no significant variations in both the groups. Glycated Haemoglobin (HbA1C) is an important parameter to assess the glycaemic control. It can be observed from Table 2 that it has reduced in the Group B but statistically it is not significant. Fasting Blood sugar levels [Figure 1] has been significantly reduced in Group B. The Biomarkers such as C - Reactive protein, Serum homocysteine and Methylmalonic acid levels significantly varied in Group B [Table 3] post supplementation with Vitamin B12. No case of Hypervitaminosis B has been observed.

DISCUSSION

The mean age and familial history for the selected participants are in accordance with the study performed earlier.[14] There is not much significant improvement in the HbA1C levels although fasting blood glucose significantly improved. This could be because both the trial groups received similar oral hypoglycemic agents.

The results obtained from our study contradicts with Jayabalan *et al.* conducted a study in 2016 titled "Vitamin B supplementation for DPN" which showed no evidence that the use of oral Vitamin B12 supplements although deficient at baseline is associated with improvement in the clinical symptoms of diabetic neuropathy.[5]

The results obtained in our study are in accordance with Andrew A. House *et al.* conducted a study in 2010 titled "influence of Vitamin B supplementation on glycemic control: Randomized controlled trial" which concluded that hyperhomocysteinemia is frequently observed in patients with diabetic neuropathy. B-Vitamin therapy (Vitamin B12) has been shown to lower the plasma concentration of homocysteine.[13]

None of the study participants developed hypervitaminosis, and no significant adverse effects were reported.

CONCLUSION

The supplementation of Vitamin B12 has significantly improved the homocysteine, MMA levels in diabetic neuropathy patients. This can be used as a suitable alternative for the available regimen.

ACKNOWLEDGMENTS

The authors are thankful to VELS Institute of Science Technology and Advanced Studies and its Management for extending their support and research facilities.

REFERENCES

1. Okada K, Tanaka H, Temporin K, Okamoto M, Kuroda Y, Moritomo H, *et al.* Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Exp Neurol 2010;222:191-203.
2. Farvid MS, Homayouni F, Amiri Z, Adelmanesh F. Improving neuropathy scores in Type 2 diabetic patients using micronutrients supplementation. Diabetes Res Clin Pract 2011;93:86-94.
3. Fonseca VA, Lavery LA, Thethi TK, Daoud Y, DeSouza C, Ovalle F, *et al.* Metanx in Type 2 diabetes with peripheral neuropathy: A randomized trial. Am J Med 2013;126:141-9.
4. Jacobs AM, Cheng D. Management of diabetic small-fiber neuropathy with combination L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate. Rev Neurol Dis 2011;8:39-47.
5. Jayabalan B, Low LL. Vitamin B supplementation for diabetic

peripheral neuropathy. Singapore Med J 2016;57:55-9.
6. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, *et al.* Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285-93.
7. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, *et al.* Vitamin B12 in metformin-treated diabetic patients: A cross-sectional study in Brazil. Rev Assoc Med Bras (1992) 2011;57:46-9.
8. Russo GT, Di Benedetto A, Magazzù D, Giandalia A, Giorda CB, Ientile R, *et al.* Mild hyperhomocysteinemia, C677T polymorphism on methylenetetrahydrofolate reductase gene and the risk of macroangiopathy in Type 2 diabetes: A prospective study. Acta Diabetol 2011;48:95-101.
9. Briani C, Dalla Torre C, Citton V, Manara R, Pompanin S, Binotto G, *et al.* Cobalamin deficiency: Clinical picture and radiological findings. Nutrients 2013;5:4521-39.
10. Chen S, Lansdown AJ, Moat SJ, Ellis R, Goringe A, Dunstan FD, Alan EJ. An observational study of the effect of metformin on B12 status and peripheral neuropathy. Br J Diabetes Vasc Dis 2012;12:189-93.
11. Ahmed MA, Muntingh G, Rheeder P. Vitamin B12 deficiency in metformin-treated Type-2 diabetes patients, prevalence and association with peripheral neuropathy. BMC Pharmacol Toxicol 2016;17:44.
12. Sato Y, Ouchi K, Funase Y, Yamauchi K, Aizawa T. Relationship between metformin use, Vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with Type 2 diabetes. Endocr J 2013;60:1275-80.
13. House AA, Eliasziw M, Cattran DC, Churchill DN, Oliver MJ, Fine A, *et al.* Effect of B-vitamin therapy on progression of diabetic nephropathy: A randomized controlled trial. JAMA 2010;303:1603-9.
14. Chowdary PR, Praveen D, Aanandhi MV. A prospective study on incidence of dyslipidemia in diabetes mellitus. Res J Pharm Tech 2017;10:431-3.

Source of support: Nil; Conflict of interest: None Declared