

Therapeutic effect of pregabalin, Vitamin B-groups and their combination on patients with diabetic peripheral poly neuropathy

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Abstract

Background and Objective: The main micro vascular complications of diabetes mellitus (DM) are neuropathy, nephropathy and retinopathy. This study was designed to evaluate and compare the effectiveness of pregabalin, Vitamin B-groups and their combination in treating patients with diabetic peripheral neuropathy (DPN).

Methods and patients: Fifteen healthy volunteers and 45 patients with diabetic neuropathy with HbA1c ranging between (6.7-12.2) % were included in this study. The patients were randomized into three groups of 15 patients each. The first group received pregabalin capsule for six weeks. The second group received B-complex tablet (B1 vitamin, B6 vitamin, B12 vitamin) twice daily for six weeks. The third group received pregabalin capsule with B-complex tablet for six weeks.

Results: Pregabalin did not significantly improve the latency of nerve conduction study in patients with DPN, but pregabalin induced a significant improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, left peroneal nerve at the fibular head and left tibial nerve at the popliteal fossa, while pregabalin could induce a significant improvement in the conduction velocity of right tibial nerve and left tibial nerve. Administration of Vitamin B-groups demonstrated a significant improvement in the latency of right tibial nerve at the ankle and left tibial nerve at the popliteal fossa of patients with DPN when compared to the pretreated patients. Furthermore, vitamin B-groups showed an improvement in the latency of left peroneal nerve at the ankle and left tibial nerve at the ankle in patients with DPN, whereas vitamin B-groups showed a significant improvement in the amplitude of right peroneal nerve at the fibular head. Daily use of

vitamin B-groups produced a significant improvement in the conduction velocity of right peroneal nerve. Administration of the combined drugs did not significantly improve the latency of left peroneal nerve at the fibular head, right and left tibial nerve at the ankle, right and left tibial nerve at the popliteal fossa in patients with DPN. Nonetheless combination of pregabalin and vitamin B-groups induced a significant improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, right peroneal nerve at the fibular head, right tibial nerve at the popliteal fossa, and left tibial nerve at the popliteal fossa of patients with DPN. Nonetheless the combined drugs could significantly recover the conduction velocity of left tibial nerve in DPN patients.

Patients receiving pregabalin for six weeks demonstrated a significant increase in the level of direct bilirubin and aspartate aminotransferase (AST). Receiving pregabalin treatment has no hazardous effect on the kidney function. Pregabalin had significantly increased the serum level of malondialdehyde of patients with diabetic peripheral neuropathy.

Conclusion: Vitamin B-groups were more efficacious than the pregabalin in improving latency of patients with DPN, while combined drugs have significantly caused greater improvement in the amplitude, than the pregabalin and vitamin B-groups. However no significant differences were found between pregabalin, Vitamin B-groups, and their combination in improving the conduction velocity in patients with DPN.

Key words: diabetic peripheral neuropathy, pregabalin, Vitamin B-groups

Introduction

Neuropathy is considered the most common micro-vascular complication of both types 1 and 2 diabetes mellitus affecting up to 50% of type 2 diabetic patients (1, 2). Increasing age, longer duration of diabetes and poor glycaemic control are well recognized risk factors for diabetic peripheral neuropathy (DPN), while cigarette smoking, hypertension, obesity, hyperlipidaemia and microalbuminuria have also been implicated as potential risk markers (3).

Diabetic peripheral neuropathy is considered a main risk factor for amputation, and hence a significant cause of morbidity in DM (4). Neuropathic disorders in diabetes can impair the function of the central, peripheral and/or autonomic nervous systems (5). The typical DPN is a chronic, symmetrical, length - dependent sensory motor polyneuropathy (6).

Distal symmetrical sensorimotor polyneuropathy is the most common type of diabetic neuropathy. It involves both small and large fibers and has an insidious onset. Typically, the most distal parts of the extremities are affected first, resulting in a stocking pattern of sensory loss (7), which is characterized by pain, numbness, and tingling in the extremities and slow nerve conduction (8). The diagnostic criteria of DPN include the presence of any abnormality (Nerve conduction velocity, amplitude, or latency) in two or more nerves on an electrophysiological test (9).

Many patients with DPN will require pharmacological treatment for painful symptoms: several agents have efficacy confirmed in published randomized controlled trials, although with the exception of Duloxetine and Pregabalin, none of the others is specifically licensed for the management of painful DPN (10). Treatments that may be beneficial for painful diabetic neuropathy include a number of antidepressants (eg, amitriptyline, duloxetine, venlafaxine) and anticonvulsants (eg, pregabalin, sodium valproate) as well as some topical agents such as capsaicin cream, lidocaine patch, and isosorbide dinitrate topical spray (11, 12). Many studies suggest the use of vitamin B-complex for the management of diabetic neuropathy. (13, 14).

Pregabalin is recommended as a first-line treatment for neuropathic pain by the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain (15). It is also recommended as a first-line treatment for DPN and central neuropathic pain by the European Federation of Neurological Societies (16) and as first-line treatment for DPN by the American Academy of Neurology (17). The course of DPN is variable. A population based study states that 10% of patients with DPN had their symptoms worsened over two years, 81% remained unchanged and 9% had improved (18).

Pregabalin 150-600 mg/day has been shown to be effective at relieving pain and reducing pain-related sleep interference in numerous well controlled trials of patients with DPN (19).

The aim of this study was to evaluate and compare the effectiveness of Pregabalin, Vitamin B-groups and their combination in treating patients with diabetic peripheral poly neuropathy, moreover the effect of pregabalin on liver function, renal function and oxidative stress.

Patients and Methods

This study was conducted at the Leila Qasm Diabetic centre, Erbil and Hawler Teaching Hospital/ Neurophysiology Unit, Erbil, from January 2013 to September 2013. Fifteen healthy volunteers and forty five patients with diabetic peripheral neuropathy were included in this study. Electromyography and biochemical tests were carried out on both the healthy volunteers and patients groups.

The control:

The control group consisted of fifteen healthy volunteers. The age of this group ranged between 20 and 55 years. All those subjects were healthy, symptom free and with no history of systemic disease or family history of diabetes mellitus. Among this group there were no histories of alcohol abuse, drug intake, or any other alternative cause of neuropathy.

The patient:

Forty five patients with diabetic neuropathy with HbA1c ranging between (6.7-12.2) %, (Mean \pm SD= 9.44 \pm 1.47) with age ranging between (30-55) years, were enrolled. This study was conducted at the Laeila Qasm Diabetic Centre, Erbil, Iraq. The ethical approval was obtained from the Ethical Committee of the College of Medicine at Hawler Medical University based on the principles of the declaration of Helsinki as revised in 2000; all patient gave informed consent.

The Inclusion criteria were predefined as follows:

- 1- Both males or females of any race over 18 years of age.
- 2- Patients with either type1 or type2 diabetes, who have been on stable anti diabetic medication regimen for at least 30 days prior to randomization.
- 3- Duration of painful diabetic peripheral neuropathy was required to be more than three months.

The criteria for exclusion were as follows:

- 1- History of smoking, alcohol consumption, and thyroid gland disorder.
- 2- Patients with any kidney disorder or any conditions that could confound assessment of pain due to diabetic peripheral neuropathy.

Study protocol:

The patients were randomized into three groups of 15 patients each

- The first group received pregabalin capsule (75mg twice daily) for six weeks.
- The second group received B-complex tablet [B1 vitamin (250mg), B6 vitamin (250mg), B12 vitamin (1mg)] twice daily for six weeks.
- The third group received pregabalin capsule (75mg twice daily) with

- The third group received pregabalin capsule (75mg twice daily) with B-complex tablet [B1 vitamin (250mg), B6 vitamin (250mg), B12 vitamin (1mg)] twice daily for six weeks. The blood samples from the first group was taken before, and six weeks after the intervention and the investigations included liver function test, renal function test, HbA1c, and malondialdehyde. Thereafter the patients were sent to Electrophysiological unit at

Hawler Teaching Hospital for performing the nerve conduction study before and six weeks after receiving the medication.

The data of Motor nerve conduction studies were analyzed using (Nicolet, Madison, WI, USA) software program.

Results

Effects of pregabalin on nerve conduction study of motor nerve of lower limb

Administration of pregabalin 75mg twice daily did not significantly improve the latency of right tibial nerve at the ankle, left tibial nerve at the ankle, right tibial nerve at the popliteal fossa and left tibial nerve at the popliteal fossa of patients with diabetic peripheral neuropathy when compared to the control group. However no significant differences were found between the latency of the control group and patients with DPN in right peroneal nerve at the ankle, left peroneal nerve at the ankle, right peroneal nerve at the fibular head and left peroneal nerve at the fibular head Table 1.

Table 1: Effects of pregabalin (75mg) on latency (ms) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy.

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve (ankle)	3.72 ± 0.06 a	4.01 ± 0.41 a	3.7 ± 0.35 a
Left peroneal nerve (ankle)	3.32 ± 0.12 a	3.98 ± 0.34 a	3.88 ± 0.32 a
Right peroneal nerve (fibular head)	10.33 ± 0.17 a	12.1 ± 0.87 a	11.13 ± 0.72 a
Left peroneal nerve (fibular head)	10.05 ± 0.21 a	12.28 ± 1.09 a	11.87 ± 1.01 a
Right tibial nerve (ankle)	3.07 ± 0.11 a	5.23 ± 0.41 b	4.78 ± 0.29 b
Left tibial nerve (ankle)	3.03 ± 0.08 a	4.78 ± 0.37 b	4.7 ± 0.44 b
Right tibial nerve (popliteal fossa)	10.22 ± 0.28 a	14.28 ± 1.18 b	14.18 ± 1.03 b
Left tibial nerve (popliteal fossa)	10.2 ± 0.2 a	14.75 ± 1.11 b	14.33 ± 1.26 b

- Mean values ± S.E are presented.
- ms= millisecond.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of pregabalin on amplitude:

The effects of pregabalin on the amplitude (mv) of the motor nerve conduction study of lower limb in patients with DPN are shown in table (2). Pregabalin induced a significant improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, left peroneal nerve at the fibular head and left tibial nerve at the popliteal fossa when compared to the pretreated patients but did not significantly reach the mean of the control healthy group. Pregabalin did not significantly improve the amplitude of right peroneal nerve at the fibular head, right tibial nerve at the ankle, left tibial nerve at the ankle and right tibial nerve at the popliteal fossa in patients with DPN when compared to the control group as shown in Table 2

Table 2: Effects of pregabalin (75mg) on amplitude (mV) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy.

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve(ankle)	5.35 ± 0.63 a	1.37 ± 0.22 c	2.67 ± 0.26 b
Left peroneal nerve (ankle)	4.86 ± 0.24 a	1.14 ± 0.23 c	2.34 ± 0.38 b
Right peroneal nerve (fibular head)	5 ± 0.35 a	1.1 ± 0.16 b	2.12 ± 0.47 b
Left peroneal nerve (fibular head)	4.29 ± 0.14 a	0.9 ± 0.1 c	2.49 ± 0.44 b
Right tibial nerve (ankle)	12.85 ± 1.21 a	1.85 ± 0.23 b	3.33 ± 1.01 b
Left tibial nerve (ankle)	9.54 ± 1.33 a	1.79 ± 0.29 b	3.73 ± 0.63 b
Right tibial nerve (popliteal fossa)	9.4 ± 0.7 a	0.95 ± 0.1 b	1.88 ± 0.22 b
Left tibial nerve (popliteal fossa)	7.53 ± 0.73 a	1.66 ± 0.36 c	3.58 ± 0.45 b

- Mean values ± S.E are presented.
- mV=millivolt
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of pregabalin on conduction velocity:

As shown in Table 3 pregabalin did not significantly improve the conduction velocity of right peroneal nerve and left peroneal nerve in patients with DPN when compared with the control group. However pregabalin could induce a significant improvement in the conduction velocity of right tibial nerve and left tibial nerve when compared to the positive control subject but did not significantly reach the mean of control healthy group.

Table 3: Effects of pregabalin (75mg) on conduction velocity (mm/s) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve	50.67 ± 0.92 a	22.78 ± 4.49 b	27.78 ± 4.04 b
Left peroneal nerve	50.71 ± 1.27 a	18.22 ± 3.77 b	21.11 ± 4.49 b
Right tibial nerve	45.25 ± 0.92 a	31.38 ± 1.36 c	35.25 ± 1.46 b
Left tibial nerve	45.57 ± 0.57 a	28.89 ± 1.51 c	35.67 ± 1.76 b

- Mean values ± S.E are presented.
- mm/s=millimeter per second
- The same letters mean that there is no significant difference.
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Effects of vitamin B-groups on nerve conduction study of motor nerve of lower limb.**Effects of vitamin B-groups on latency:**

Table 4 shows that vitamin B-groups did not significantly improve the latency of left peroneal nerve at the fibular head and right tibial nerve at the popliteal fossa in patients with DPN when compared with the control group. Vitamin B-groups demonstrated a significant improvement in the latency of right tibial nerve at the ankle and left tibial nerve at the popliteal fossa of patients with DPN when compared to the pretreated patients, but did not significantly reach the mean of control healthy group. Furthermore, vitamin B-groups showed an improvement in the latency of left peroneal nerve at the ankle and left tibial nerve at the ankle in patients with DPN, however, no significant difference was found between post-treated patients and each of healthy subjects and the pre-treated group. Moreover no significant differences were observed between the latency of control group and patients with DPN in right peroneal nerve at the ankle, right peroneal nerve at the fibular head, Table 4.

Table 4: Effects of vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on latency (ms) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve (ankle)	3.72 ± 0.06 a	4.26 ± 0.3 a	3.66 ± 0.23 a
Left peroneal nerve (ankle)	3.32 ± 0.12 a	4.34 ± 0.23 b	3.96 ± 0.33 ab
Right peroneal nerve (fibular head)	10.33 ± 0.17 a	10.67 ± 0.23 a	10.44 ± 0.34 a
Left peroneal nerve (fibular head)	10.05 ± 0.21 a	11.82 ± 0.27 b	10.98 ± 0.4 b
Right tibial nerve (ankle)	3.07 ± 0.11 a	4.93 ± 0.26 c	3.98 ± 0.09 b
Left tibial nerve (ankle)	3.03 ± 0.08 a	4.64 ± 0.39 b	3.91 ± 0.48 ab
Right tibial nerve (popliteal fossa)	10.22 ± 0.28 a	14.68 ± 0.64 b	13.44 ± 0.84 b
Left tibial nerve (popliteal fossa)	10.2 ± 0.2 a	13.7 ± 0.54 c	12.3 ± 0.44 b

- Mean values ± S.E are presented.
- ms= millisecond.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of vitamin B-groups on amplitude:

The effect of vitamin B-groups including vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg) on amplitude (mv) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy is shown in Table 5. Daily uses of vitamin B-groups did not significantly improve the amplitude of patients with DPN in right peroneal nerve at the ankle, left peroneal nerve at the ankle, left peroneal nerve at the fibular head, right tibial nerve at the ankle, left tibial nerve at the ankle, right tibial nerve at the popliteal fossa, left tibial nerve at the popliteal fossa when compared with the control group. Vitamin B-groups showed a significant improvement in the amplitude of right peroneal nerve at the fibular head when compared to the pretreated patients but did not significantly reach the mean of the healthy group.

Table 5: Effects of vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on amplitude mV of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy.

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve (ankle)	5.35 ± 0.63 a	1.46 ± 0.17 b	2.33 ± 0.36 b
Left peroneal nerve (ankle)	4.86 ± 0.24 a	1.4 ± 0.15 b	1.89 ± 0.25 b
Right peroneal nerve (fibular head)	5 ± 0.35 a	1.01 ± 0.09 c	2 ± 0.35 b
Left peroneal nerve (fibular head)	4.29 ± 0.14 a	1.61 ± 0.17 b	1.68 ± 0.27 b
Right tibial nerve (ankle)	12.85 ± 1.21 a	2.24 ± 0.29 b	4.71 ± 1.1 b
Left tibial nerve (ankle)	9.54 ± 1.33 a	2.11 ± 0.48 b	3.66 ± 0.98 b
Right tibial nerve (popliteal fossa)	9.4 ± 0.7 a	± 0.19 1.1 b	1.96 ± 0.51 b
Left tibial nerve (popliteal fossa)	7.53 ± 0.73 a	1.76 ± 0.42 b	1.83 ± 0.3 b

- Mean values ± S.E are presented.
- mV= millivolt.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of vitamin B-groups on conduction velocity:

Table 6 demonstrates that the vitamin B-groups have no significant effects in the improvement of conduction velocity in left peroneal nerve and left tibial nerve of patients with DPN when compared with the control group.

However Vitamin B-groups produced a significant improvement in the conduction velocity of right peroneal nerve when compared with the positive control subjects but did not significantly reach the mean of the healthy group. As far as the right tibial nerve is concerned, it appears that vitamin B-groups improved the conduction velocity in patients with DPN but no significant difference was established between post-treated patients and each of healthy subjects and pre-treated group.

Table 6: Effects of vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on conduction velocity (mm/s) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy.

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve	50.67 ± 0.92 a	36.38 ± 1.07 c	40.63 ± 1.48 b
Left peroneal nerve	50.71 ± 1.27 a	34.22 ± 3.13 b	36.78 ± 1.92 b
Right tibial nerve	45.25 ± 0.92 a	29.86 ± 4.07 b	37.43 ± 3.54 ab
Left tibial nerve	45.57 ± 0.57 a	33.14 ± 1.01 b	35 ± 1.93 b

- Mean values \pm S.E are presented.
- mm/s=millimeter per second
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of combination of pregabalin and vitamin B-groups on nerve conduction study of motor nerve of the lower limb.

Effects of pregabalin and vitamin B-groups on the latency:

Table 7 shows that there was improvement in the latency of left peroneal nerve at the ankle however it was not significant. The combined drugs did not significantly improve the latency of left peroneal nerve at the fibular head, right and left tibial nerve at the ankle, and right and left tibial nerve at the popliteal fossa in patients with DPN. No significant differences were found between the healthy group and patients with DPN concerning right peroneal nerve at the ankle and right peroneal nerve at the fibular head.

Table 7: Effects of pregabalin (75mg) and vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on latency (ms) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve (ankle)	3.72 \pm 0.06 A	4.12 \pm 0.53 a	4.08 \pm 0.42 a
Left peroneal nerve (ankle)	3.32 \pm 0.12 a	4.72 \pm 0.64 ab	5.47 \pm 0.49 b
Right peroneal nerve (fibular head)	10.33 \pm 0.17 a	11.52 \pm 0.95 a	11.28 \pm 0.91 a
Left peroneal nerve (fibular head)	10.05 \pm 0.21 a	13.43 \pm 0.88 b	13.22 \pm 0.82 b
Right tibial nerve (ankle)	3.07 \pm 0.11 a	4.73 \pm 0.41 b	5.27 \pm 0.47 b
Left tibial nerve (ankle)	3.03 \pm 0.08 a	5.23 \pm 0.46 b	5.22 \pm 0.54 b
Right tibial nerve (popliteal fossa)	10.22 \pm 0.28 a	17.15 \pm 0.59 b	17.18 \pm 0.56 b
Left tibial nerve (popliteal fossa)	10.2 \pm 0.2 a	13.82 \pm 1 b	14.22 \pm 0.99 b

- Mean values \pm S.E are presented.
- ms= millisecond.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of pregabalin and vitamin B-groups on the amplitude:

Table 8 shows that combination of pregabalin and vitamin B-groups induced a significant improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, right peroneal nerve at the fibular head, right tibial nerve at the popliteal fossa, and left tibial nerve at the popliteal fossa of patients with DPN when compared with the positive control subjects but did not significantly reach the mean of the control healthy group. In left peroneal nerve at the fibular head the combination of pregabalin and vitamin B-groups significantly increased the mean value of amplitude of diabetic patients. However, the combined drugs have no significant effects on the amplitude of right tibial nerve at the ankle and left tibial nerve at the ankle in patients with DPN.

Table 8: Effects of pregabalin (75mg) and vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on amplitude mV of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve (ankle)	5.35 ± 0.63 a	1.04 ± 0.16 c	2.99 ± 0.23 b
Left peroneal nerve (ankle)	4.86 ± 0.24 a	1.33 ± 0.17 c	3.14 ± 0.36 b
Right peroneal nerve (fibular head)	5 ± 0.35 a	2.32 ± 0.34 c	3.57 ± 0.43 b
Left peroneal nerve (fibular head)	4.29 ± 0.14 a	± 0.13 1.01 b	3.68 ± 0.39 a
Right tibial nerve (ankle)	12.85 ± 1.21 a	1.97 ± 0.54 b	3.95 ± 0.52 b
Left tibial nerve (ankle)	9.54 ± 1.33 a	1.46 ± 0.42 b	3.73 ± 0.47 b
Right tibial nerve (popliteal fossa)	9.4 ± 0.7 a	1.07 ± 0.37 c	3.42 ± 0.31 b
Left tibial nerve (popliteal fossa)	7.53 ± 0.73 a	1.41 ± 0.39 c	2.93 ± 0.29 b

- Mean values ± S.E are presented.
- mV= millivolt.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

Effects of pregabalin and vitamin B-groups on conduction velocity:

The effects of the combined drugs on the conduction velocity in DPN patients and in comparison with the healthy group are shown in Table 9. Daily administration of both pregabalin and vitamin B-groups non-significantly improved the conduction velocity of right peroneal nerve, left peroneal nerve, and right tibial nerve in DPN patients. Nonetheless the combined drugs could significantly recover the conduction velocity of left tibial nerve in DPN patients.

Table 9: Effects of pregabalin (75mg) and vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)] on conduction velocity (mm/s) of motor nerve conduction study of the lower limb in patients with diabetic peripheral neuropathy

Site	Control Healthy subjects (n=15)	Pre-treatment (n=15)	Post-treatment (n=15)
Right peroneal nerve	50.67 ± 0.92 a	24.56 ± 4.57 b	29.89 ± 3.22 b
Left peroneal nerve	50.71 ± 1.27 a	31.8 ± 3.17 b	38.1 ± 3.15 b
Right tibial nerve	45.25 ± 0.92 a	32.33 ± 1.17 b	34.83 ± 1.01 b
Left tibial nerve	45.57 ± 0.57 a	32 ± 2.46 b	40.86 ± 1.47 a

- Mean values ± S.E are presented.
- mm/s=millimeter per second
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at $p < 0.05$

The net effects of pregabalin, vitamin B-groups, and their combination on the motor nerve of the lower limb after six weeks treatment.

The net effects of pregabalin, vitamin B-groups and their combination on the latency:

The comparison of the net differences in the studied latency before and after six weeks treatment between pregabalin, vitamin B-groups, and their combination is shown in Table 10. Vitamin B-groups have significantly greater improvements in the latency of right and left tibial nerve at the popliteal fossa than that of pregabalin and combined drugs. No significant differences were found between the net effects of pregabalin, vitamin B-groups, and their combination in the latency of right peroneal nerve at the ankle, or left tibial nerve at the ankle. Both vitamin B-groups and combined drugs have significantly better effects than those of pregabalin in the latency of right peroneal nerve at the fibular head. However vitamin B-groups and pregabalin have higher affinity in reducing latency than the combined drugs of left peroneal nerve at the ankle, left peroneal nerve at the fibular head, and right tibial nerve at the ankle.

Table 10: The net differences in the studied latency (ms) before and after six weeks treatment between pregabalin (75mg), vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)], and their combination (n=45).

Site	Pregabalin	Vitamin b-groups	Combined drugs
Right peroneal nerve (ankle)	-0.37 ± 0.09 a	-0.6 ± 0.26 a	-0.03 ± 0.24 a
Left peroneal nerve (ankle)	-0.1 ± 0.43 ab	-0.39 ± 0.27 a	0.75 ± 0.27 b
Right peroneal nerve (fibular head)	-0.06 ± 0.28 a	-0.23 ± 0.29 b	-0.23 ± 0.08 b
Left peroneal nerve (fibular head)	-0.42 ± 0.15 ab	-0.83 ± 0.17 a	-0.22 ± 0.09 b
Right tibial nerve (ankle)	-0.45 ± 0.45 a	-0.96 ± 0.27 a	0.53 ± 0.09 b
Left tibial nerve (ankle)	-0.08 ± 0.21 ab	-0.73 ± 0.24 a	-0.02 ± 0.13 b
Right tibial nerve (popliteal fossa)	-0.1 ± 0.25 b	-1.23 ± 0.3 a	0.03 ± 0.08 b
Left tibial nerve (popliteal fossa)	-0.42 ± 0.11 b	-1.4 ± 0.28 a	-0.4 ± 0.17 c

- Mean values ± S.E are presented.
- ms= millisecond.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at p < 0.05

The net Effects of pregabalin, vitamin B-groups and the combination of both drugs on the amplitude:

The comparison of the net differences in the studied amplitude before and after six weeks treatment between (pregabalin, vitamin B-groups, and their combination) is shown in Table 11. The combined drugs showed significantly a greater improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, left peroneal nerve at the fibular head and right tibial nerve at the popliteal fossa in patients with DPN, than that of pregabalin and vitamin B-groups. Combined drugs and pregabalin have slightly greater affects in the amplitude of right peroneal nerve at the fibular head, right tibial nerve at the ankle, and left tibial nerve at the ankle than that of vitamin B- groups but the result was not significant. Both the combined drugs and pregabalin produced significantly greater improvements of the amplitude in left tibial nerve at the popliteal fossa than the vitamin B-groups.

Table 11: The net differences in the studied amplitude (mV) before and after six weeks treatment between pregabalin (75mg), vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)], and their combination (n=45)

Site	Pregabalin	Vitamin B groups	Combined drugs
Right peroneal nerve (ankle)	1.3 ± 0.22 ab	0.86 ± 0.31 a	1.94 ± 0.61 b
Left peroneal nerve (ankle)	1.2 ± 0.41 ab	0.49 ± 0.3 a	1.81 ± 0.34 b
Right peroneal nerve (fibular head)	± 0.46 1.02 a	0.99 ± 0.29 a	1.24 ± 0.3 a
Left peroneal nerve (fibular head)	1.59 ± 0.43 b	0.09 ± 0.18 a	2.68 ± 0.4 c
Right tibial nerve (ankle)	1.48 ± 0.98 a	0.47 ± 0.99 a	1.98 ± 0.69 a
Left tibial nerve (ankle)	1.94 ± 0.62 a	1.54 ± 0.87 a	2.24 ± 0.55 a
Right tibial nerve (popliteal fossa)	0.93 ± 0.21 a	0.66 ± 0.53 a	2.35 ± 0.42 b
Left tibial nerve (popliteal fossa)	1.92 ± 0.42 b	0.07 ± 0.65 a	1.51 ± 0.32 b

- Mean values ± S.E are presented.
- mV= millivolt.
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at p < 0.05

The net effects of pregabalin, vitamin B-groups and combination of both drugs on conduction velocity:

Table 12 shows that no significant difference were found among (pregabalin, vitamin B-groups, and their combination) on the conduction velocity of the right peroneal nerve, left peroneal nerve, and right tibial nerve. While combined drugs has shown a significantly larger improvement in the conduction velocity of the left tibial nerve than either of pregabalin and vitamin B-groups.

Table 12: The net differences in the studied conduction velocity (mm/s) before and after six weeks treatment between pregabalin (75mg), vitamin B-groups [vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg)], and their combination (n=45)

Site	Pregabalin	Vitamin B groups	Combined drugs
Right peroneal nerve	5 ± 0.97 a	4.25 ± 1.62 a	5.33 ± 1.93 a
Left peroneal nerve	2.89 ± 0.9 a	3.11 ± 1.75 a	6.3 ± 1.98 a
Right tibial nerve	3.88 ± 1.14 a	7.57 ± 4.93 a	2.5 ± 0.56 a
Left tibial nerve	6.78 ± 1.82 ab	1.86 ± 2.72 a	10.29 ± 1.74 b

- Mean values ± S.E are presented.
- mm/s= millimeter per second
- The same letters mean that there is no significant difference.
- The different letters mean there is a significant difference at p < 0.05

Effects of pregabalin on biochemical test (liver function test, renal function test, and serum malondialdehyde).**Effects of pregabalin on liver function test:**

Administration of pregabalin capsule (75mg) twice daily significantly increased the serum level of direct bilirubin ($P=0.02$) and aspartate aminotransferase ($P=0.048$). However pregabalin did not significantly change the serum level of indirect bilirubin, serum alkaline phosphates, and alanin aminotransferase Table 13.

Following six weeks of treatment with pregabalin (75 mg twice daily) there were no significant changes found in the renal function test of DPN patients. (Table 13)

Use of pregabalin for six weeks significantly increased serum level of malondialdehyde of patients with DPN (Table 13).

Table 13: Effects of pregabalin on liver function test, renal function test and malondialdehyde $\mu\text{m/l}$. n=15

Parameters	Normal value	Pre-treatment	Post-treatment
Direct bilirubin mg/100ml	0.1-0.2	0.24 \pm 0.04	0.33 \pm 0.04 *
Indirect bilirubin mg/100ml	0.2-0.4	0.33 \pm 0.08	0.35 \pm 0.06
ALP IU/L	100-290	218.67 \pm 41.57	293.33 \pm 50.57
AST U/ml	<40	21.17 \pm 2.98	24.17 \pm 3 *
ALT U/ml	<45	18.67 \pm 3.65	21.33 \pm 2.63
Blood urea mg/100ml	10-50	30 \pm 3.47	32.33 \pm 3.41
Serum creatinine mg/100ml	0.6-1.3	0.89 \pm 0.05	0.87 \pm 0.06
Serum malondialdehyde $\mu\text{m/l}$	0.1-10	12.19 \pm 2.14	14.95 \pm 2.09 *

* ($P<0.05$) when compared to control group

Discussion

The peripheral nerve in diabetic patients is characterized by progressive nerve fiber loss with pan-modal fiber size pattern (20, 21). In particular, the nerve fiber degeneration is length-dependent and conspicuous in distal portion. It has recently been shown that small fibers are preferentially affected in early stages of diabetic patients followed by the involvement of large fibers related to reduced nerve conduction velocity (22, 23). The progressive nerve fiber loss may be attributed to ongoing axonal degeneration or severe demyelination (24). The axonal loss leads to lower amplitudes, and demyelination causes prolonged latency and slow conduction velocity (25).

Effects of drug on nerve conduction study of motor nerve of lower limb:

The result of this study showed that administration of pregabalin 75mg twice daily for six weeks did not produce significant effect on the latency of nerve conduction study in patients with diabetic peripheral neuropathy.

These results support the results of Arezzo et al (2008) who reported that pregabalin 300 mg twice daily had no statistically significant effect on nerve conduction in patients with DPN (26).

The effect of pregabalin (75mg twice daily) on amplitude of NCS after six weeks were significant improvement of amplitude of right and left peroneal nerve at the ankle, left peroneal nerve at the fibular head, left tibial nerve at the popliteal fossa. This result is inconsistent with the finding of Tucker (2006) who showed that 600mg of pregabalin daily for 13 week had no clinically significant changes in the nerve conduction parameters including amplitude and conduction velocity (27). However in right peroneal nerve at the fibular head, left and right tibial nerve at the ankle and right tibial nerve at the popliteal fossa pregabalin had no significant effects on the amplitude of NCS. Hence, this part of our results is in agreement with the finding of Tucker (2006) (27).

The results of the present study showed that use of pregabalin for six weeks caused a significant improvement in the conduction velocity of right and left tibial nerve. This finding is incomparable with the study of Mathur et al (2014) as they observed that administration of pregabalin 600mg/d for 12 weeks did not produce significant effects on the amplitude and conduction velocity (28).

In this study, vitamin B-groups vitamin B1 (250mg), vitamin B6 (250mg), vitamin B12 (1mg) showed a significant improvement in latency of right tibial nerve at the ankle and left tibial nerve at the popliteal fossa after six weeks of therapy. The same dose of vitamin B groups significantly increased conduction velocity of right peroneal nerve. These improvements in the latency and conduction velocity most probably is attributed to the role of vitamin B-groups in formation and synthesis of myelin, which is the protective coating that insulates the nerve (29), also vitamin B-groups can activate chemical signals that help nerves' survival and regeneration (30, 31, 32).

However vitamin B groups could not significantly change the amplitude of NCS of right and left peroneal nerve at the ankle, left peroneal nerve at the fibular head, right and left tibial nerve at the ankle, and right and left tibial nerve at the popliteal fossa. This indicated that vitamin B groups showed no affect in the development of axon of nerve fibers (25, 29).

The results of the present study showed that the combination of pregabalin and vitamin B groups has no significant effects in improving latency of right and left peroneal nerve at the ankle and at the fibular head, right and left tibial nerve at the ankle and at the popliteal fossa and conduction velocity of right peroneal nerve, left peroneal nerve, and right tibial nerve. However the combined drugs could significantly improve the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, right peroneal nerve at the fibular head, right tibial nerve at the popliteal fossa, and left tibial nerve at the popliteal fossa, and left peroneal nerve at the fibular head. These interesting results might be attributed to medication non-adherence as reported by NCPIE (2000) which states that non-adherence can take a variety of forms, including not having a prescription filled, taking an incorrect dose, taking medication at the wrong time, forgetting to take doses, or stopping therapy too soon (33).

The net effects of pregabalin, vitamin B-groups, and their combination on the motor nerve of lower limb after six weeks treatment.

The effect of vitamin B-groups on latency of right and left tibial nerve at the popliteal fossa, right peroneal nerve at the fibular head, left peroneal nerve at the ankle, left peroneal nerve at the fibular head, and right tibial nerve at the ankle is better than the pregabalin and their combination. Vitamin B-groups' effectiveness in improving latency might be contributed to the potential role of vitamin B-groups in improving neuropathy and

maintaining the health of small nerves in lower extremities (34, 35, 36).

Combined drugs has significantly caused greater improvement in the amplitude of right peroneal nerve at the ankle, left peroneal nerve at the ankle, left peroneal nerve at the fibular head, and right tibial nerve at the popliteal fossa than that of pregabalin and vitamin B-groups, however in right peroneal nerve at the fibular head, right tibial nerve at the ankle and left tibial nerve at the ankle combined drugs have slightly greater effect on amplitude than vitamin B-groups and pregabalin but the result was deemed to be non-significant. Donger and Swami (2013) concluded that vitamin B-groups play an important role in the regeneration of the myelin sheath (37), and the pregabalin may restore the function of nerve. It is well known that vitamin B1 (thiamin) is contributed to the synthesis of neurotransmitter acetylcholine and it facilitates the conduction of nerve impulses through the peripheral nerves. Thiamin is also instrumental in producing myelin, while vitamin B12 or cobalamin is required for the manufacture of healthy myelin (29). There were no significant effects of pregabalin, vitamin B-groups and their combination found in conduction velocity.

Effect of pregabalin on biochemical test (liver function test, renal function test, and serum malondialdehyde):

In the present study, patients receiving pregabalin (75 mg twice daily) demonstrated a significant increase in the level of direct bilirubin and aspartate aminotransferase. However, pregabalin intake did not significantly change the serum level of indirect bilirubin, serum alkaline phosphates, and alanin aminotransferase. These results support the findings of (Sendra et al, 2011) who reported that administration of pregabalin 25 mg twice daily for 14 days significantly induced an elevation in liver enzymes (AST, ALT and total bilirubin) of a 54 -year old patient (38).

Following six weeks of receiving pregabalin treatment (75 mg twice daily), there was no significant change noted in the level of blood urea and serum creatinine when compared to pre-treatment levels. Hence, it can be concluded that pregabalin has no hazardous effect on the kidney functions according to the above findings.

The use of pregabalin for six weeks had significantly increased the serum level of malondialdehyde in this study. It was found by Kay et al, (2012) that pregabalin induces hypoxia and increases endothelial cell proliferation in mice, daily administration produced a significant increase in an immunohistochemical stain causing hypoxia in the liver of mice (39). The oxidative stress that occurs during cellular hypoxia is likely involved in this disorder. Indeed, the reactive species were demonstrated to inhibit active substances, modulate the signaling of intracellular pathways, and mediate enzymes activation, which are known to play a critical role in the geneses and/or the outcomes of this disorder (40)

Conclusion

Vitamin B-groups were more efficacious than the pregabalin in improving latency of patients with DPN. While combined drugs have significantly caused greater improvement in the amplitude than that of pregabalin and vitamin B-groups. However no significant differences were found between pregabalin, Vitamin B-groups, and their combination in improving the conduction velocity in patients with DPN.

References

- 1- Boulton A, Malik R, Arezo J, Sosenko J. Diabetic somatic neuropathies. *Diabetes Care*; 2003; 27:1458-86.
- 2- Daousi C, Mac F, Woodward A, Nurmikko T, Bundred P. Chronic painful peripheral neuropathy in an urban community: a controlled comparison of people with and without diabetes. *Diabet Med*, 2004; 21(9):976-982.
- 3- Boulton A, Cavanagh P, Rayman G. The foot in diabetes. Fourth edition. John Wiley & Sons Ltd, Hoboken, 2006.
- 4- Jeffcoate W, Harding K. Diabetic foot ulcers. *Lancet*, 2003; 361(9368):1545-1551.
- 5- American Diabetes Association, American Academy of Neurology. Consensus statement: report and recommendations of the San Antonio conference on diabetic neuropathy. American Diabetes Association American academy of neurology. *Diabetes Care*, 11(7):592-597. 1988.
- 6- Dyck P, Kratz K, Karnes J, Litchy W, Klein R, Pach J. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology*; 1993; 43:817-24.
- 7- Melton L, Dyck P. Epidemiology: In *Diabetic Neuropathy*. 2 edition edition. W.B. Saunders, Philadelphia. 1999
- 8- Gregg E, Sorlie P, Paulose-Ram R. Prevalence of lower-extremity disease in the US adult population ? 40 years of age with and without diabetes. 1999-2000 National Health and Nutrition Examination Survey. *Diabetes Care*; 2004; 27:1591-1597.
- 9- Arimura A, Deguchi T, Sugimoto K, Nakamura T. Intraepidermal nerve fiber density and nerve conduction study parameters correlate with clinical staging of diabetic polyneuropathy. *Diabetes research and clinical practice*; 2013; 99; 24 - 29.
- 10- Boulton A, Malik R, Arezzo J, Sosenko J. Diabetic somatic neuropathies (Technical Review). *Diabetes Care*; 2004; 27:1458-1486.
- 11- Finnerup N, Otto M, McQuay H. Algorithm for neuropathic pain treatment an evidence based proposal. *Pain*; 2005; 118: 289-305.
- 12- Finnerup N, Grydehoj J, Bing J. Levetiracetam in spinal cord injury pain a randomized controlled trial. *Spinal Cord*; 2009; 47: 861-867.
- 13- Murphy S, White K, Park S. Multivitamin-multimineral supplements' effect on total nutrient intake. *Am J Clin Nutr* 85: 2007; 280S.
- 14- Andrew A, Misha E, Daniel C, David N, Matthew J, Adrian F, George K. Effect of B-Vitamin Therapy on Progression of Diabetic Nephropathy. 2010; 303(16):1603-1609.
- 15- Dworkin R, O'Connor A, Audette J. Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin Proc*; 2010; 85(3 Suppl): S3-14.
- 16- Attal N, Cruccu G, Baron R. European Federation of Neurological Societies EFNS guidelines on the pharmacological treatment of neuropathic pain. *Eur J Neurol*; 2010; 17(9): 1113-e88.
- 17- Bril V, England J, Franklin G. Evidence-based guideline: Treatment of painful diabetic neuropathy: Report of the American Academy of Neurology, the American. 2011
- 18- Diabetes Control and Complications Trial (DCCT) Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *N Engl J Med*; 1993; 329:977-986.
- 19- Freeman R, Durso-Decruz E, Emir B. Efficacy, safety, and tolerability of pregabalin treatment for painful diabetic peripheral neuropathy: findings from seven randomized, controlled trials across a range of doses. *Diabetes Care*; 2008; 31(7): 1448-54.
- 20- Yagihashi Y. Pathology and pathogenetic mechanisms of diabetic neuropathy, *Diabetes Metab. Rev.* 1995; 11; 193-225.
- 21- Dyck P, Giannini C. Pathologic alterations in the diabetic neuropathies of humans: a review, *J. Neuropathol. Exp. Neurol.* 1996; 55; 1181-1193.
- 22- Dyck P, Larson T, O'Brien P, Velosa J. Patterns of quantitative sensation testing of hypoesthesia and hyperalgesia are predictive of diabetic polyneuropathy: a study of three cohorts. Nerve growth factor study group, *Diabetes Care*, 2000; 23; 510-517.
- 23- Sumner C, Sheth S, Griffin J, Cornblath D, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance, *Neurology*; 2003; 60; 108-111.
- 24- Yagihashi S, Yamagishi Sh, Wada R (2007). Pathology and pathogenetic mechanisms of diabetic neuropathy: Correlation with clinical signs and symptoms. Department of pathology, Hiro saki university school of medicine, 5 Zaifu-cho, Hiro saki; 2007; 036-8562.
- 25- Azhary H, Farooq M, Bhanushali M, Majid A, Kassab M. *Peripheral Neuropathy: Differential Diagnosis and Management*; 2010; 81(7):887-892.
- 26- Arezzo J, Rosenstock J, Lamoreaux L, Pauer L. Efficacy and safety of pregabalin 600 mg/d for treating painful diabetic peripheral neuropathy: A double-blind placebo-controlled trial. *BMC neurology*; 2008; 8:33.
- 27- Tucker M. Pain and headache. *Clinical neurology news*; 2006; p.12. www.jfponline.com.z
- 28- Mathur S, Mckay G, Fisher M. Drug notes pregabalin. *Practical diabetes*; 2014; 31:1.p 40-41a.
- 29- Beers M. Thiamin deficiency and vitamin B12 deficiency. *The Merck manual of diagnosis and therapy*, 2006; 18th edition.
- 30- Jian-bo L and Cheng-ya W. The Preventive Efficacy of Methylcobalamin on Rat Peripheral Neuropathy Influenced by Diabetes via Neural IGF-1 Levels. *Nutritional Neuroscience*; 2010; 13(2): 79-86.

- 31- Okada K and Tanaka H. Methylcobalamin Increases Erk1/2 and Akt Activities through the Methylation Cycle and Promotes Nerve Regeneration in a Rat Sciatic Nerve Injury Model. *Experimental Neurology*; 2010; 222: 191-203.
- 32- Mizukami H and Ogasawara S. Methylcobalamin Effects of Diabetic Neuropathy and Nerve Protein Kinase C in Rats. *European Journal of Clinical Investigation*; 2011; 41(4): 442-450.
- 33- National Council on Patient Information and Education (NCPIE). The other drug problem: Statistics on medicine use and compliance. www.talkaboutrx.org; 2000
- 34- Walker M and Morris L (2010). Improvement of Cutaneous Sensitivity in Diabetic Peripheral Neuropathy with Combination L-Methylfolate, 2010
- 35- Jacobs A and Cheng D. Management of Diabetic Small-Fiber neuropathy with Combination L-Methylfolate, Methylcobalamin, and Pyridocyl 5'-Phosphate. *Reviews of Neurological Diseases*; 2011 8(1/2): 1-9.
- 36- Fonseca V and Lavery L. Metax in Type 2 Diabetes with Peripheral Neuropathy: A Randomized Trial. *The American Journal of Medicine*; 2013; 126, 141-149.
- 37- Donger Y and Swami O. Sustained-release with methylcobalamin in neuropathic pain: an Indian real-life experience. *International Journal of general medicine*, 2013; 6; 413-417.
- 38- Sendra J, Junyent T, Pellicer M. Pregablin-induced hepatotoxicity. *Ann Pharmacother*; 2011; 45:e32.
- 39- Kay C, Jon C, Dennis M, Michael L, Christopher S, et al. Pregabalin induces Hepatic Hypoxia and Increases Endothelial cell proliferation in mice; process inhibited by Dietary vitamin E supplementation. *Toxicological Sciences*. 2012; 128(1, pp. 42-56.
- 40- Pialoux V and Mounier R. Hypoxia-Induced Oxidative Stress in Health Disorders. *Oxidative Medicine and Cellular Longevity*. vol 2012, Article ID 940121, p. 2.