

Comparative Study of Vitamin B Complex Combined with Alpha Lipoic Acid versus Vitamin B Complex in Treatment of Diabetic Polyneuropathy in Type 2 Diabetic Patients

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Abstract

Objective: Diabetic Polyneuropathy (DPN) represents a major health problem as it increases morbidity affecting patients' quality of life. Vitamin B frequently is used for treating DPN. ALPHA Lipoic Acid (ALA) seems to delay or reverse DPN. The present study compared the effect of Vitamin B complex combined with ALA versus Vitamin B in treatment of DPN in Egyptian type 2 diabetic patients.

Methods: Forty type 2 diabetic patients with DPN of whom twenty received once-daily oral doses of ALA (600 mg) in combination with Vitamin B complex and the other twenty only received vitamin B complex for 12 weeks. Patients were evaluated by Michigan Neuropathy Screening Instrument (MNSI) questionnaire and Nerve Conduction Studies (NCS). Blood samples were collected for determination of Insulin Resistance and lipid abnormalities. All the previous assessments were performed at baseline and at week 12.

Results: There was a significant reduction after 12 weeks of supplementation in vitamin B complex plus Alpha Lipoic Acid (ALA) group using MNSI questionnaire ($p=0.001$) and not in vitamin B complex group. NCS demonstrated that 12 out of 16 patients in vitamin B complex plus ALA group showed improvement in at least one property measured compared with only 6 out of 16 in vitamin B complex group ($p=0.0732$). The influence of the combination on the progression of diabetes with regards to insulin resistance and lipid profile showed no advantage over Vitamin B complex alone.

Conclusion: Combined therapy of DPN with ALA and Vitamin B complex, orally for 12 weeks improves the symptoms of neuropathy (MNSI) with a similar trend in NCS.

Keywords: DPN; Diabetic polyneuropathy; Alpha lipoic acid; Vitamin B complex; Type 2 diabetes

Introduction

Diabetic Neuropathy (DN), one of diabetes microvascular complications, consists of various syndromes with different anatomical distributions, clinical courses, and possibly different underlying pathogenesis. Neuropathy can be either diffuse or focal. The diffuse neuropathies i.e., distal symmetrical sensorimotor polyneuropathy (DPN) and Diabetic Autonomic Neuropathy (DAN) are common, usually chronic, and often progressive [1].

DPN causes pain or loss of feeling in the toes, feet, legs, hands, and arms. It represents a major health problem and it is responsible for impaired quality of life [2]. Positive symptoms include burning, tingling ('pins and needles' or paraesthesia), shooting or lancing (stabbing) that patients experience in stocking-glove distribution. Many of these symptoms are attributable to small-fiber neuropathy, which often precedes large fiber dysfunction [3]. Negative

symptoms include loss of sensation and loss of strength suggestive of large fiber damage [4]. DPN is, therefore, associated with substantial morbidity including: Depression, susceptibility to foot or ankle fractures, ulceration and lower-limb amputations [5].

Pathophysiological pathways of DPN originate mainly from oxidative stress. Elevated intracellular levels of glucose lead to Advanced Glycation End-products (AGE) formation and polyol pathway activation, resulting in subsequent formation of reactive oxygen species [2]. The only proven treatment strategy is strict glycemic control [6]. While an effective cornerstone of therapy, near-normoglycemia is not always possible to achieve or maintain [7]. For symptomatic treatment of DPN, Tricyclic Antidepressants (TCAs) or anticonvulsants may be used [8]. Given the role of oxidative stress in DPN progression, antioxidants have been proven to be effective in preventing or delaying the onset of DPN such as acetyl-L-carnitine, taurine [9] and Alpha Lipoic Acid (ALA) the only fat and water soluble antioxidant [10].

In humans, ALA is synthesized by the liver and other tissues but is also found naturally in the diet [11]. High concentrations of ALA are

found in animal food sources with extensive metabolic activity, such as heart, liver and kidney. Spinach, broccoli, tomato, garden pea, brussels sprout and rice bran are among non-animal sources of ALA [12]. ALA contains two thiol (sulfur) groups, which may be oxidized or reduced Dihydrothiolipoic Acid (DHLA). Both ALA and DHLA are potent antioxidants, with the latter regenerating other factors such as vitamins C and E in addition to raising glutathione intracellularly. Furthermore, both ALA and DHLA function as free radical scavengers [13]. In neuroblastoma cell cultures, ALA has been associated with the sprouting of neurites [14]. It also increases blood flow, glucose uptake, and metabolism in peripheral nerves along with Nerve Conduction Velocity (NCV) [15]. Generally, ALA is a safe drug. Its main untoward effects have included occasional gastrointestinal complaints that comprised nausea, vomiting, abdominal discomfort and diarrhea [16]. Despite its qualities as a safe, available, inexpensive antioxidant, ALA is not widely used, in part, due to incomplete knowledge of its efficacy and adverse effects [13].

Many randomized controlled trials (RCTs) have been conducted to assess the effect of intravenous alpha lipoic acid for the treatment of DPN. A meta-analysis including four trials (ALADIN I, ALADIN III, SYDNEY, NATHAN II) provide evidence that parenteral treatment with ALA (600 mg/day i.v.) over 3 weeks, significantly improves positive neuropathic symptoms and neuropathic deficits in patients with DPN [17]. In another study, intravenous ALA (300-600 mg per day, for two to four weeks) was safe and could significantly improve positive neuropathic symptoms as well as nerve conduction velocity but the evidence was mainly of poor quality [18].

Benefits of oral treatment with ALA were studied in SYDNEY 2. A 5 weeks trial showed that Oral treatment with ALA for 5 weeks improved neuropathic symptoms and deficits in patients with DPN, an oral dose of 600 mg once daily provide the optimum risk-to-benefit ratio while effect on electrophysiological parameters was not reported [19].

It should also be noted that Vitamin B complex is frequently used for treating peripheral neuropathy due to its availability and affordability but its efficacy is not reproducible [20]. Vitamin B12 (cobalamin) is an important biofactor promoting various metabolic cascades for cellular activity and survival in both haematopoietic and nervous tissues [21]. In particular, methyl-base-attached cobalamin (methylcobalamin; MC) is shown to have threefold stronger affinity for nerve tissues compared with other types of cobalamins [22].

As previously found [23] serum vitamin B12 levels were significantly lower in 20 type 2 diabetic patients compared to 20 age matched controls. Supplementation of 1,500 mcg/day methylcobalamin for two months resulted in improved vibratory perception thresholds and heart rate variability (a sign of improvement of autonomic neuropathy) in the diabetic group [23]. Supplementation of vitamin B12 may benefit DPN by correcting a deficiency. Not only diabetes can result in vitamin B12 deficiency but also metformin, an oral antihyperglycemic agent used to treat type 2 diabetes, may cause vitamin B12 deficiency [24]. In a double blinded study, patients with DPN showed statistical improvement in the somatic and autonomic symptoms with regression of signs of diabetic neuropathy while motor and sensory nerve conduction studies showed no statistical improvement after 4 months of methylcobalamin intake [25].

The present pilot trial is, therefore, carried out to compare the effect of ALA when combined with Vitamin B complex versus Vitamin B complex for the treatment of DPN in Egyptian type 2 diabetic patients.

Subjects and Methods

Study subjects

Screening of the study subjects was done at the outpatient Diabetes and Endocrinology Clinic of Ain Shams University Hospital through a period of 12 months from October 2015 to October 2016. Patients of both genders between the age of (18 and 70) who fulfilled the following criteria were considered eligible as subjects:

- Diagnosed as having type 2 diabetes as per ADA criteria [26].
- Patients showing clinical signs of DPN verified clinically by a consultant neurologist and confirmed by score ≥ 7 on MNSI questionnaire [27], with abnormal nerve conduction measurement enough to define DPN according to American Academy of Neurology [28].
- Blood sugar controlled for at least 2 months prior study from their files.
- Patients with neuropathy of non-diabetic origin (organochlorine pesticides exposure, drugs (such as cisplatin, taxol, corticosteroids, sulfa drugs, etc.), severe neurologic diseases (Parkinson's disease, epilepsy, etc.), malignancy, myxedema and chronic illnesses: renal dysfunction (serum creatinine >1.5 mg%); hepatic abnormality (serum total bilirubin >2.5 mg%), existing chronic hepatitis B infection or HIV infection) or patients using drugs with possible influence on the study results (antidepressants, anticonvulsants, opiates, mexiletine, capsaicin, neuroleptics, aldose reductase inhibitors, antioxidants, and particularly methylcobalamin, pyridoxine and other B complex preparations) were excluded.
- Patients with BMI of 40 kg/m² or more and those pregnant or breastfeeding were also excluded.

Patients who met the eligibility criteria were randomly selected. In total, 40 patients were enrolled. Written informed consent was obtained from each of the participants after they had received a full explanation of the study. The study was approved by the Institutional Ethics Committee at Ain Shams University and was conducted in accordance with the ethical principles set forth in the Helsinki Declaration of 1975 as revised in 1983.

Study design and observation items

This was a prospective, open-label controlled study with a 12-weeks period. After screening, forty patients were randomly allocated into two groups:

- Vitamin B complex plus alpha lipoic acid (ALA) group AB (n=20) received once-daily oral doses of ALA (600 mg, Thiotacid 600, Evapharma, Egypt) in combination with Vitamin B complex (B1+B6+B12, 150 mg+100 mg+1 mg, respectively, Neurovit, European Egyptian Pharm. Ind., Egypt), two tablets per day.
- Vitamin B complex group B (n=20) received only Vitamin B complex (B1+B6+B12) two tablets per day for the same period.

All patients were clinically monitored for any adverse reactions such as nausea, vomiting, abdominal discomfort, and diarrhea every 4 weeks [16]. Compliance was verbally confirmed by consumption of the tablets as per the treatment schedule. All the patients were counselled about diet control and oral hypoglycemic agent.

A 15-items questionnaire form of Michigan Neuropathy Screening Instrument (MNSI) consisting of yes/no questions was applied to all patients, before and at the end of treatment, of those, 13 items assess

symptoms of diabetic peripheral neuropathy, 1 item assesses peripheral vascular disease, and 1 item assesses general asthenia [27]. The questionnaire inquires about positive (pain, temperature sensation, tingling) and negative (numbness) sensory symptoms, cramps and muscle weakness, foot ulcers or cracks and amputation [29]. All items on the questionnaire were coded as 0 for a negative response and 1 for a positive response (negative responses on items 7 and 13 counted as 1 point). A higher MNSI score is associated with a more severe DN [29]. A score of seven or more positive responses on the MNSI questionnaire confirm neuropathy [27]. This questionnaire was translated to the Arabic language by “Fouad Nemah Bureau for Translation and Authorship” in Cairo and revised by a consultant neurologist. Efficacy assessment variable was the change in MNSI questionnaire score from baseline to week 12.

Assessment of Nerve Conduction Studies (NCS) was also performed for both sides (right and left lower limbs) using the Keypoint Medtronic EMG machine. Motor nerve conduction parameters including Compound Muscle Action Potential (CMAP) amplitudes, distal latency and motor conduction velocity were measured in common peroneal and posterior tibial nerves. Sensory conduction studies included measurement of peak latency and amplitude of SNAPs. Stimulating and recording parameters were kept constant in all subjects to ensure adequate uniformity in study procedure. The change in week 12 NCS from baseline was considered improvement in DPN according to the criteria shown in Table 1.

	Nerve properties	conduction
Motor nerves	Amplitude	≥1 mV
	Latency	≥1 m/s
	Conduction velocity	≥10 m/s
Sensory nerves	Amplitude	≥5 μV
	Latency	≥1 m/s
	Conduction velocity	≥10 m/s

Table 1: Reference values for improvement in NCS.

Neurological assessments were carried out by trained and certified neurologist who was blinded to randomization of patients during clinical assessment and NCS

The following laboratory investigations were done at baseline and week 12: Fasting plasma glucose level, fasting Insulin level using Insulin ELISA Kit [30], insulin resistance quantification by calculation of HOMA IR [31], fasting lipid profile including total cholesterol [32], triglycerides [33], HDL [34] and LDL [35].

Statistical analysis

Data are reported as mean ± SD values, median, or number with percentage in parentheses for categorical variables. For significance tests, baseline clinical and laboratory characteristics within-group comparisons were performed with the paired Student’s t test or Wilcoxon signed-rank test. Intergroup comparison was performed with unpaired Student’s t test, Fisher’s exact test or Chi square test where appropriate. For all tests, a value of P ≤ 0.05 was considered statistically significant. GraphPad Prism (version-3) was used for statistical analyses.

Results

Demographic parameters

A total of 8 subjects discontinued during the treatment period, and 32 patients completed the trial. The baseline characteristics of the study subjects are shown in Table 2. Mean age of all patients was (53.9 ± 8.2), 62.5% were female with a mean diabetes duration of (6.6 ± 5.46) years. After randomization, there was no significant difference among the groups for any of the parameters assessed except for the height and this may be due to the relative higher number of males in group B. There were no adverse events reported during treatment period.

	B (n=16)	AB (n=16)	P value
Gender%			
Male [n (%)]	8 (50)	4 (25)	0.2734
Age in years			
Mean ± SD	55.69 ± 9.67	52.13 ± 6.22	0.2246
Range	41.0–70.0	39.0–63.0	
Weight in kg			
Mean ± SD	87.25 ± 12.90	80.88 ± 13.10	0.1757
Range	62.0–108.0	58.0–101.0	
Height in meter			
Mean ± SD	1.66 ± 0.04	1.60 ± 0.07	0.0134*
Range	1.60–1.75	1.40–1.740	
BMI (kg/m²)			
Mean ± SD	31.75 ± 4.66	31.67 ± 5.96	0.9634
95% CI	29.27–34.24	28.49–34.84	
Years with diabetes			
Mean ± SD	7.06 ± 5.67	6.15 ± 5.38	0.6422
Range	1.00–25.00	0.16–20.00	
HbA1C %			
Mean ± SD	6.21 ± 1.04	6.43 ± 1.3	0.6028
95% CI	5.65–6.76	5.73–7.12	

Table 2: Patient demographic characteristics. Results are shown as number (%) or mean ± SD of base line measurements of 16 type 2 diabetic patients in group B (Vitamin B complex) or group AB (vitamin B complex combined with alpha lipoic acid). Results are compared using unpaired Student’s t test except for the Gender (Fisher’s exact test). 95% CI: 95% Confidence Interval. BMI: Body Mass Index. HbA1C: Glycated Hemoglobin. *Indicates significant difference.

The effect of supplementation with vitamin B complex without (B) or with alpha lipoic acid (AB) on diabetic polyneuropathy (DPN)

Michigan neuropathy screening instrument questionnaire score: Michigan Neuropathy Screening Instrument (MNSI) questionnaire was used as a subjective measure of improvement. The baseline score for MNSI questionnaire for groups B and AB was similar ($p=0.5077$). After 12 weeks of therapy, a significant reduction with AB was found ($p=0.0001$) and not in the other group ($p=0.0547$). Median changed from 9 to 7 in AB group and no change in the median before and after treatment for group B. This resulted in a significant reduction in the median score between groups after 12 weeks ($p=0.0023$). Furthermore, 6 out of 16 patients in AB group compared with 0/16 in B group ($p=0.0177$) showed improvement by scoring less than 7 (cut-off value) (Figure 1).

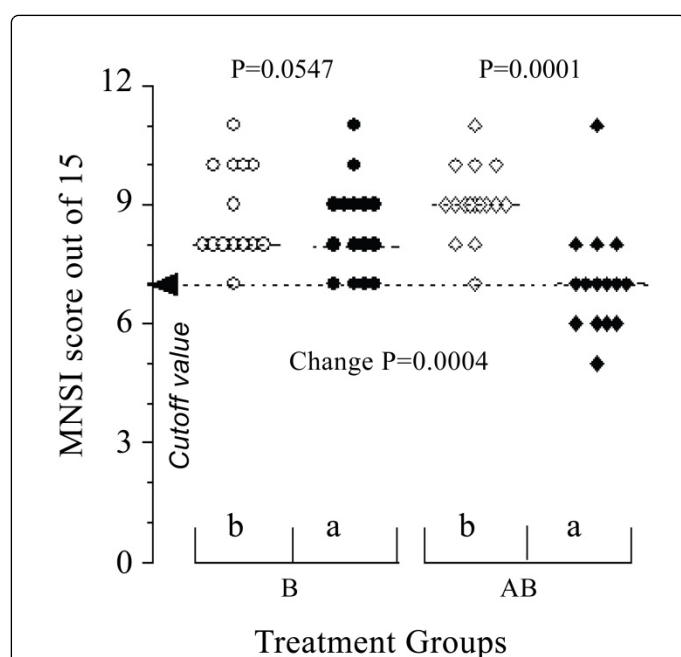


Figure 1: Scatter diagram represents Michigan Neuropathy Screening Instrument (MNSI) questionnaire score of type 2 diabetic patients before (b) and 12 weeks after (a) oral treatment with Vitamin B complex without (B) or with alpha lipoic acid (AB). Median of each group is shown as transverse line. Results are compared using Wilcoxon signed rank test (b vs. a) and Mann Whitney (change).

Effect of supplementation on nerve function

Detailed electrophysiological measurements: When detailed electrophysiological nerve conduction was assessed based on the change from base line as improved (I), worsened (W), or with no change (N) as shown in Figure 2, tibial nerve measurements showed a significant improvement in the amplitude ($p=0.0153$) as well as latency ($p=0.0159$) of the left leg (L) of patients but not the right (R) in the combination group (AB) compared with B group (Figure 2A). This was shown by the increase in the number of patients improved and a decrease in those with no effect or worsened. The same applies for peroneal nerve with a significant improvement in amplitude

($p=0.0425$) and conduction velocity ($p=0.0121$) in the left side for patients in group AB (Figure 2A). Similar measurements of the right side didn't reach a significant difference. For sensory nerve evaluation (Figure 2B), improvement was found in the amplitude ($p=0.0283$) and latency ($p=0.0213$) of sural nerve of the left side and the amplitude of the right side in the combination group (AB) compared with B group.

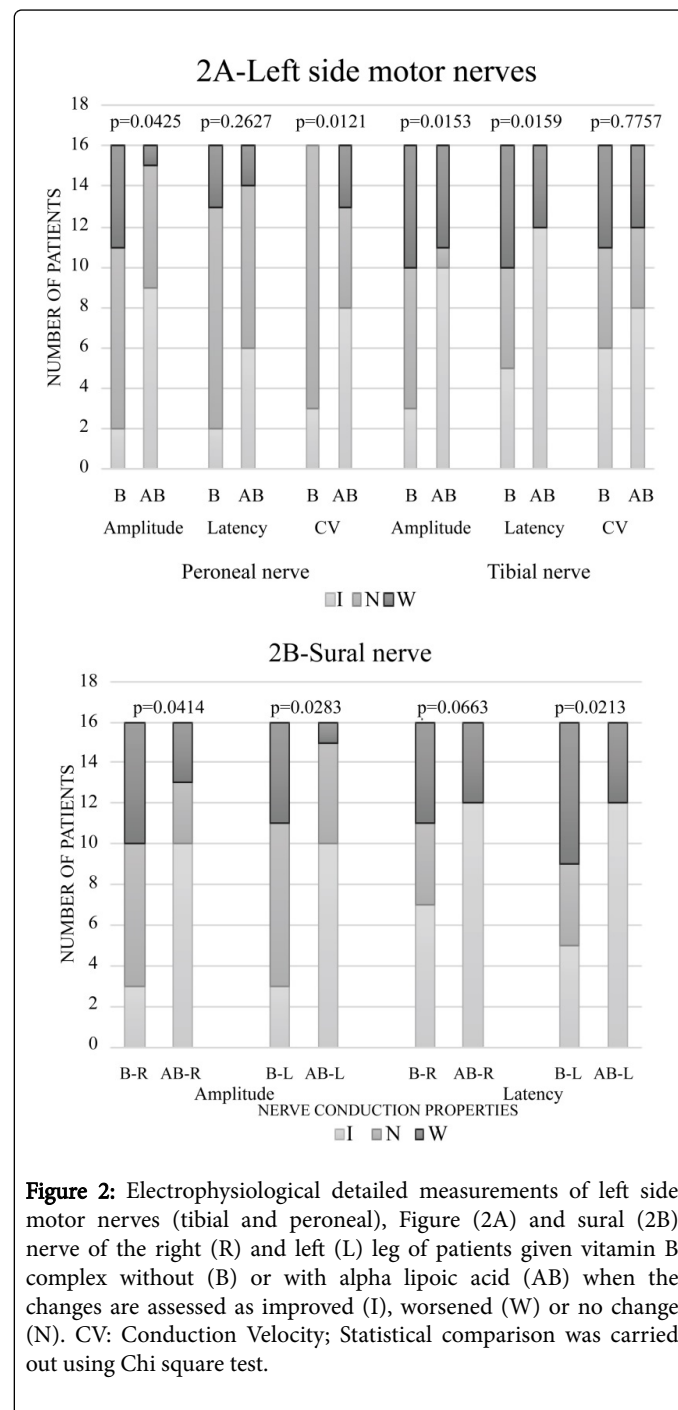
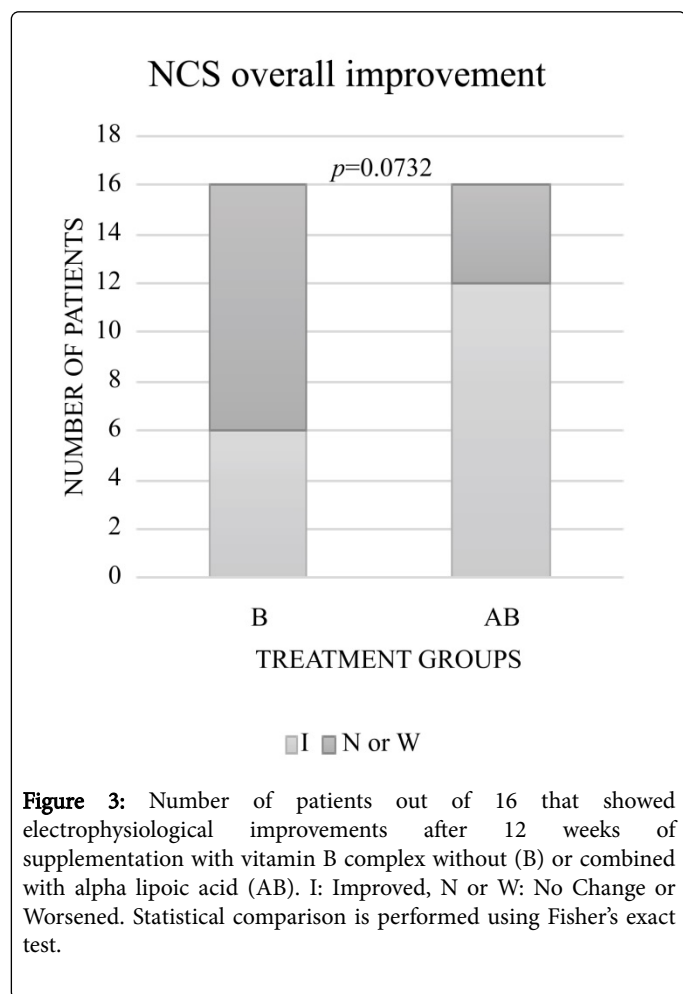


Figure 2: Electrophysiological detailed measurements of left side motor nerves (tibial and peroneal), Figure (2A) and sural (2B) nerve of the right (R) and left (L) leg of patients given vitamin B complex without (B) or with alpha lipoic acid (AB) when the changes are assessed as improved (I), worsened (W) or no change (N). CV: Conduction Velocity; Statistical comparison was carried out using Chi square test.

Overall effect of supplementation on electrophysiological measurements: Electrophysiological measures of nerve conduction, has demonstrated that 12 out of 16 patients in group AB showed improvement in at least one property (amplitude, latency, velocity) of any right or left lower limb nerves (peroneal, tibial, and sural)

according to the criteria listed in Table 1, compared with only 6 out of 16 in the other group ($p=0.0732$) as demonstrated in Figure 3.



Effect of supplementation on insulin resistance

No statistically significant difference was found between patients in group B compared to those in group AB at baseline as well as after treatment in fasting plasma glucose level. Fasting insulin level and HOMA-IR showed, however, significant difference between patients in group B compared to those in group AB at baseline ($p=0.002$, $p=0.005$) respectively. On the other hand, there was no statistically significant difference within groups in fasting plasma glucose or insulin level as well as HOMA-IR (Table 3).

Effect of supplementation on lipid abnormalities

No statistically significant difference was found between patients in group B compared to those in group AB at baseline and even after treatment as well as within groups with regards to Total Cholesterol, Triglycerides High Density Lipoprotein cholesterol (HDL-C) or Low density lipoprotein cholesterol (LDL-C) (Table 3).

Discussion

DPN represents a major health problem and is responsible for impaired quality of life [2]. The prevalence of neuropathy in diabetic patients is about 30%, whereas up to 50% of patients will certainly

develop neuropathy during the course of the disease [36]. As glycemic variability leads to oxidative stress, antioxidants such as ALA are valuable therapeutic option for DPN [37]. ALA can potently regenerate other antioxidants such as vitamin C, vitamin E and glutathione through redox cycling [38].

Parameter	B			AB			P value of Change (AB vs. B)
	b	a	P value (a vs. b)	b	a	P value (a vs. b)	
FPG (mg/dL)	138.7 ± 83.95	189.61 ± 93.52	0.0821	179.59 ± 106.37	224.74 ± 155.03	0.246	0.638
FIL (mU/L)	2.22 ± 2.43	4.38 ± 7.47	0.1365	6.03 ± 5.47	4.34 ± 5.87	0.3624	0.118
HOMA- IR	0.78 ± 1.26	1.87 ± 3.65	0.0992	2.67 ± 2.62	2.68 ± 4.7	0.9938	0.127
TC (mg/dL)	263.94 ± 94.51	242.62 ± 89.56	0.4707	224.7 ± 83.43	242.24 ± 82.98	0.4538	0.356
TG (mg/dL)	191.86 ± 99.18	149.13 ± 57.73	0.1124	141.25 ± 67.89	145.13 ± 50.17	0.853	0.462
HDL-C (mg/dL)	61 ± 18.27	57.38 ± 22.8	0.453	53.25 ± 24.61	54.13 ± 17.13	0.8964	0.88
LDL-C (mg/dL)	164.56 ± 74.21	155.42 ± 78.25	0.6874	143.2 ± 53.04	159.09 ± 66.52	0.3445	0.266

Table 3: Laboratory investigations to assess supplementations effect on insulin resistance and lipid profile. Results are expressed as mean ± SD. Paired t test was used to assess change within each group, p value ≤ 0.05 is considered significant. Group (B): Control group received Vitamin B complex. Group (AB): Test group received vitamin B complex combined with ALA. b: before treatment or baseline, a: after treatment, FPG: Fasting Plasma Glucose, FIL: Fasting Insulin Level, HOMA-IR: Homeostasis Model Assessment Of Insulin Resistance, TC: Total Cholesterol, TG: Triglycerides, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol.

In the present trial, the effect of oral supplementation with ALA, 600 mg daily dose, on DPN was evaluated when given with vitamin B complex compared with vitamin B complex alone. For that purpose, MNSI questionnaire was used, as well as electrophysiological nerve function measurements of the lower limb. The study continued for 12 weeks.

Improvement of the symptoms of neuropathy (MNSI questionnaire) was evident in the AB group compared with B group. ALA was previously found to improve positive neuropathic symptoms when given intravenously [18] and orally [13]. The present study used NCS as a parameter to assess the nerve function for both sensory and motor peripheral nerves. Findings of our study suggest that the combination of ALA and Vitamin B complex was effective in improving nerve function parallel to symptoms improvement. In a previous study,

patients with DPN showed statistical improvement in the somatic and autonomic symptoms with regression of signs of diabetic neuropathy after 4 months of methylcobalamin intake [25].

The influence of this supplementation on Insulin Resistance (IR) and lipid abnormalities has also been studied. There was no significant difference observed between the groups with regards to Fasting plasma glucose, Fasting insulin level or HOMA-IR in contrary to previous findings by Kamenova [39] who found that a 4 weeks oral treatment with ALA, 600 mg twice daily, significantly increased peripheral insulin sensitivity in patients with DM2 to a level almost similar to that of subjects with normal glucose tolerance. When the effect on lipid abnormalities was assessed, results didn't show any advantage for the combination over lipid profile, though the efficacy of ALA on dyslipidemia was different in other studies [40].

The present study has a number of limitations, including an open-label design and small sample size. An open-label study design does not include blinding which may introduce assessment bias. Large, randomized, double-blind studies for a longer duration are required to explore the benefits of combining ALA and Vitamin B complex for DPN treatment.

Conclusion

The present study shows that combined therapy of DPN with ALA and Vitamin B complex, orally for 12 weeks improves the symptoms of neuropathy (MNSI) with a similar trend in NCS. To our knowledge, the present preliminary trial might be the only one to show the clinical and electrophysiological effects of ALA/Vitamin B complex oral combination in type 2 Egyptian diabetic patients. Future studies should focus on repeating a trial on a larger number of patients with double blinded, controlled design and for longer duration of treatment.

Author Disclosure Statement

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References

1. Edwards JL, Vincent AM, Cheng HT, Feldman EL (2008) Diabetic neuropathy: mechanisms to management. *Pharmacol Ther* 120: 1-34.
2. Hosseini A, Abdollahi M (2013) Diabetic neuropathy and oxidative stress: therapeutic perspectives. *Oxid Med Cell Longe* 2013: 1-15.
3. Rutkove SB, Veves A, Mitsa T (2009) Impaired distal thermoregulation in diabetes and diabetic polyneuropathy. *Diabetes Care* 32: 671-676.
4. Yagihashi S, Yamagishi S, Wada R (2007) Pathology and pathogenetic mechanisms of diabetic neuropathy: correlation with clinical signs and symptoms. *Diabetes Res Clin Pract* 77: S184-S189.
5. Singh R, Kishore L, Kau N (2014) Diabetic peripheral neuropathy: current perspective and future directions. *Pharmacol Res* 80: 21-35.
6. Bailey CJ (2000) Potential new treatments for type 2 diabetes. *Trends Pharmacol Sci* 21: 259-265.
7. Ziegler D (2008) Painful diabetic neuropathy: treatment and future aspects. *Diabetes Metab Res Rev* 24: S52-S57.
8. Collins SL, Moore RA, McQuay HJ, Wiffen P (2000) Antidepressants and anticonvulsants for diabetic neuropathy and postherpetic neuralgia: a quantitative systematic review. *J Pain Symptom Manage* 20: 449-458.
9. Shakher J, Stevens MJ (2011) Update on the management of diabetic polyneuropathies. *Diabetes Metab Syndr Obes* 4: 289-305.
10. Vallianou N, Evangelopoulos A, Koutalas P (2009) Alpha-lipoic Acid and diabetic neuropathy. *Rev Diabet Stud* 6: 230-236.
11. Foster TS (2007) Efficacy and safety of alpha-lipoic acid supplementation in the treatment of symptomatic diabetic neuropathy. *Diabetes Educ* 33: 111-117.
12. Packer L, Witt EH, Tritschler HJ (1995) Alpha-Lipoic acid as a biological antioxidant. *Free Radic Biol Med* 19: 227-250.
13. McIllduff CE, Rutkove SB (2011) Critical appraisal of the use of alpha lipoic acid (thioctic acid) in the treatment of symptomatic diabetic polyneuropathy. *Ther Clin Risk Manag* 7: 377-385.
14. Dimpfel W, Spuler M, Pierau FK, Ulrich H (1990) Thioctic acid induces dose-dependent sprouting of neurites in cultured rat neuroblastoma cells. *Dev Pharmacol Ther* 14: 193-199.
15. Stevens MJ, Obrosova I, Cao X, Van Huysen C, Greene DA (2000) Effects of DL-alpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. *Diabetes* 49: 1006-1015.
16. Rathmann W, Haastert B, Delling B, Gries FA, Giani G (1998) Postmarketing surveillance of adverse drug reactions: a correlational study approach using multiple data sources. *Pharmacoepidemiol Drug Saf* 7: 51-57.
17. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA (2004) Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med* 21: 114-121.
18. Han T, Bai J, Liu W, Hu Y (2012) A systematic review and meta-analysis of alpha-lipoic acid in the treatment of diabetic peripheral neuropathy. *Eur J Endocrinol* 167: 465-471.
19. Ziegler D, Ametov A, Barinov A, Dyck PJ, Gurieva I, et al. (2006) Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial. *Diabetes Care* 29: 2365-2370.
20. Ang CD, Alviar MJ, Dans AL, Bautista-Velez GG, Villaruz-Sulit MV, et al. (2008) Vitamin B for treating peripheral neuropathy. *Cochrane Database Syst Rev* CD004573.
21. Bourre JM (2006) Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. *J Nutr Health Aging* 10: 377-385.
22. Metz J (1992) Cobalamin deficiency and the pathogenesis of nervous system disease. *Annu Rev Nutr* 12: 59-79.
23. Yoshioka K, Tanaka K (1995) Effect of methylcobalamin on diabetic autonomic neuropathy as assessed by power spectral analysis of heart rate variations. *Horm Metab Res* 27: 43-44.
24. Liu KW, Dai LK, Jean W (2006) Metformin-related vitamin B12 deficiency. *Age Ageing* 35: 200-201.
25. Yaqub BA, Siddique A, Sulimani R (1992) Effects of methylcobalamin on diabetic neuropathy. *Clin Neurol Neurosurg* 94: 105-111.
26. ADA (2015) Standards of medical care in diabetes-2015 abridged for primary care providers. *Clin Diabetes* 33: 97-111.
27. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, et al. (1994) A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care* 17: 1281-1289.
28. England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, et al. (2005) Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology* 64: 199-207.
29. Herman WH, Pop-Busui R, Braffett BH, Martin LC, Cleary AP, et al. (2012) Use of the Michigan neuropathy screening instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the diabetes control and complications trial/epidemiology of diabetes interventions and complications. *Diabet Med* 29: 937-944.
30. Bowsher RR, Wolny JD, Frank BH (1992) A rapid and sensitive radioimmunoassay for the measurement of proinsulin in human serum. *Diabetes* 41: 1084-1090.
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell

-
- function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419.
32. Roeschlau P, Bernt E, Gruber W (1974) Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem* 12: 226.
33. McGowan MW, Artiss JD, Strandbergh DR, Zak B (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-542.
34. Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem* 23: 882-884.
35. Friedwald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502.
36. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL, et al. (2012) Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol* 11: 521-534.
37. Papanas N, Ziegler D (2014) Efficacy of alpha-lipoic acid in diabetic neuropathy. *Expert Opin Pharmacother* 15: 2721-2731.
38. Miranda-Massari JR, Gonzalez MJ, Jimenez FJ, Allende-Vigo MZ, Duconge J, et al. (2011) Metabolic correction in the management of diabetic peripheral neuropathy: improving clinical results beyond symptom control. *Curr Clin Pharmacol* 6: 260-273.
39. Kamenova P (2006) Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. *Hormones (Athens)* 5: 251-258.
40. Hamano Y (2006) Effects of dietary lipoic acid on plasma lipid, in vivo insulin sensitivity, metabolic response to corticosterone and in vitro lipolysis in broiler chickens. *Br J Nutr* 95: 1094-1101.