Vitamins B therapy in Regeneration of Peripheral Neuropathy Associated with Lipid Profile

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Vitamins B therapy in Regeneration of Peripheral Neuropathy Associated with Lipid Profile

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Abstract. Objective: To investigate the role of serum lipid profile during the regeneration of experimental crush peripheral nerve injury to rats. Besides, to investigate which type of vitamin B therapy is better to accelerate the regeneration processes.Methods: Seventy-five male albino rats male, aged between 10 to 12 weeks were equally divided into 5 treated groups: B1, B6, B12, Tri-B complex and normal saline. Rats of therapeutic groups were administered according to the grouping and lasted to 45 consecutive days, and every 5 rats were sacrificed by euthanizing at 15th, 30th and 45th days. At dissection, sciatic nerve samples are taken from the site of crushed lesion and immersed in fixed formaldehyde 10%. 05 to 7 µm thick paraffin sections were stained by H&E and blood sample for lipid profile analysis.Results: The result showed no significant differences in serum lipid profile between therapeutic groups and control was found. Histological changes were shown on 15th days as, diffuse degeneration of the myelinated fibers with a marked vacuolization of some nerve fiber, numerous atrophic disoriented fibers with a large number of mononuclear phagocytes infiltration and degenerative debris. On 45th days there is a clear normal orientation of collagen fiber with very little debris and no vacuolization of the nerve. Morphometric measurements showed the myelin sheath thickness was increased significantly on the 30th and 45th days as compared with the control group. The result showed vitamin B12 is better in acceleration to nerve regeneration B12 groups showed better regenerative rate among other groups.Conclusion: no changes in serum lipid profile on experimental animals during regeneration processes. Vitamin B12 is better for enhancing the regenerative process.

KEYWORDS: Regeneration; Peripheral Neuropathy ; Vitamin B; Lipid profile

INTRODUCTION

During the last decades, there are increasing shreds of evidence of involvement of the vitamins B complex in the peripheral neuropathy, in the acceleration of nerve repair, both in the recovery of nerve function and enhancement of nerve regeneration.[1,2] These findings are proved that experimental peripheral nerve injury leads to significant variations in nerve tissue levels of the vitamins B especially in the acute phase of the injury, leading to the supplementation with vitamins B complex in the acute period of peripheral nerve injury which gives rise to a beneficial result in accelerating nerve regeneration [1]. In addition to the neurological role of vitamin B deficiencies, vitamins B1, B6, and B12 have been shown to exert anti-inflammatory and analgesic effects in experimental animal models. [3-6] The experimental crushing of a sciatic nerve stimulates numerous pathways, which lead to additional increasing production of the oxidants. Stresses due to crushing nerve reduced important minerals, vitamins, and nutrients from the body which may result in certain diseases of the peripheral nervous system. [1,4,7] . Okada and Tanaka and Sun et al. [8,9] mention that the vitamin B complex and vitamin B12 plays a crucial role in fat metabolism and act as an anti-stress factor. Vitamin B12 has been shown synergistic acceleration to the rate of regeneration and functional recovery of injured sciatic nerves through increasing BDNF expression. Although, another vitamin B has anti-stress and antioxidant properties of vitamin B1 and B6 respectively.[10-12] Treatment with different types of vitamin B has been shown to increase the number of Schwann cells, myelinated nerve fibers and the diameter of axons, thereby promoting the regeneration of the

injured peripheral nerve.[1,13] It has also been shown that the use of antioxidant vitamin B1, B6, B12 is either reduces the post-injury nerve dysfunction and improves or accelerate nerve regeneration in healthy sciatic nerve crushed. [1,2,9] Therefore, this study was undertaken to determine the effects of each type of vitamins B on the rate of regeneration in crush sciatic nerve. Severe vitamin B12 deficiency causes an increase in the plasma cholesterol and triglycerides levels in female mice. [14] also dietary excess of vitamin B6 intake reduces serum total cholesterol levels, but not serum HDL and total lipid levels in rats. [15] Previous studies used epinephrine as a stress factor or other stress factors that lead to an increase of atherogenic LDL and total plasma cholesterol. [16-,18] The aim of the present study is to investigate the role of the serum lipid profile measurement during vitamins B therapy of rats experimentally subjected to sciatic nerves crush. Morphometric methods were also used in many experimental models of peripheral neuropathies and regeneration studies.[19,20] Application of vitamin B complex or vitamin B12 has been shown to increase the number of Schwann cells, myelin sheaths and the diameter of axons, and thereby promote the regeneration of myelinated nerve fibers and the proliferation of Schwann cells. [13] Our previous study.[21] suggested that the Vitamins B12 is better in the regeneration of crushed sciatic nerve than vitamin B1, and B6. In this study, we try to confirm the previous result by using other methods in morphometric measurements of crushed sciatic nerves during therapy by different types of vitamins B and the assessment the morphological characteristics of sciatic nerve regeneration after standardized crush injury as a control for further studies belong to this object.

MATERIALS AND METHODS

Animal husbandry

The study protocol was approved by the Basra medical college/ Animal House. Seventy-five male albino rats aged between 10-12 weeks of an average weight of 200 gm were used in this study. They were housed in stainless steel cages in an air-conditioned house. Rats were fed normal pellet was given to the rat and supplied with clean water. Rats were equally divided into 5 groups as follows: Group A has administrated i.m injection with 1ml normal saline as a control group. Group B was given i.m injection of vitamin B1 (thiamin hydrochloride 180mg/kg/day). Group C given i.m injection of vitamin B6 (pyridoxine 180mg/kg/day) as The National Research Council (NRC) this dose was chosen based on.[22] Group D was given i.m injection with vitamin B12 (Methylcobalamin 1mg/kg/day) according to [23]. Group E was co-administrated i.m injection of Tri-B (B1, B6 & B12), (20 mg/kg/day) according to [18]. Rats of therapeutic groups were administered for 15^{th}, 30^{th} and 45^{th} consecutive days.

Surgical technique

Rats were anesthetized by using an anesthetic solution such as chloroform. The rats were placed in prone position and sterilization of the skin at the posterior aspect of the right thigh with 10% povidone-iodine after shaving. The right sciatic nerve of all rats was exposed at the mid-thigh level and crushed at the location 1 cm from the sciatic notch for 60 seconds with Kocher's hemostatic forceps (5-10mm) as reported previously by many investigators. [5,23,24] Crush level was marked on the muscle by 2/0 non-absorbable silk suture to be sure for taking the sample.

Diagnostic samples

Blood samples were collected in sterile tubes by using small bore needle by heart puncture after anesthetized the animals by using ketamine hydrochloride 90mg/kg and xylazine hydrochloride 5mg/kg[23]. Serum was separated from blood clotting then centrifuged for 15 minutes at the rate of 4000 rpm. Cholesterol and triglyceride were measured by a quantitative method[25]. LDL cholesterol was determined by using polyvinyl sulfates method [26]. High-Density Lipoprotein (HDL-c) was determined by the precipitation of chylomicrons by[27]. Sciatic nerve samples of all rats were taken at 15^{th}, 30^{th} and 45^{th} days of crushing. Nerve section (5 to 7µm) were cut including the crash site and then fixed in formalin for histological examination. After embedding in paraffin, 7 µm thick slices were taken and stained by Hematoxylin and Eosin. The morphometric measurement images were acquired via a digital camera (LEICA, DMC, 2900, Germany) and analyzed with an IBM/PC. For the myelin thickness measurements, a modern Leica light microscope 1000X magnification provided with a camera (0.5X), as well as a 10X computerized magnification which provided a high-resolution image[20]. We try to measure the means ± SEM error of means of (10) nerves from every three sizes of rat sciatic nerve (small, medium and large) randomly to avoid any bias. All data were evaluated by using Student-Neyman-Keuis Post Hoc test and one-way analysis of variables and type 1 error rate was accepted as 0.05 in the statistical calculation.

RESULTS

Biochemical analysis

The assay results for serum TC, HDL-c, LDL-c, VLDL-c and TG of the rats that injected with vitamin B1, B6, B12 and Tri-B with a normal group at 30th days are shown in fig. 1. The current study found no significant differences between the mean values of the serum lipid profile of therapeutic groups and control group, also no significant differences were found between the therapeutic groups in each period(15th, 30th and 45th days) of the experiment table 1.

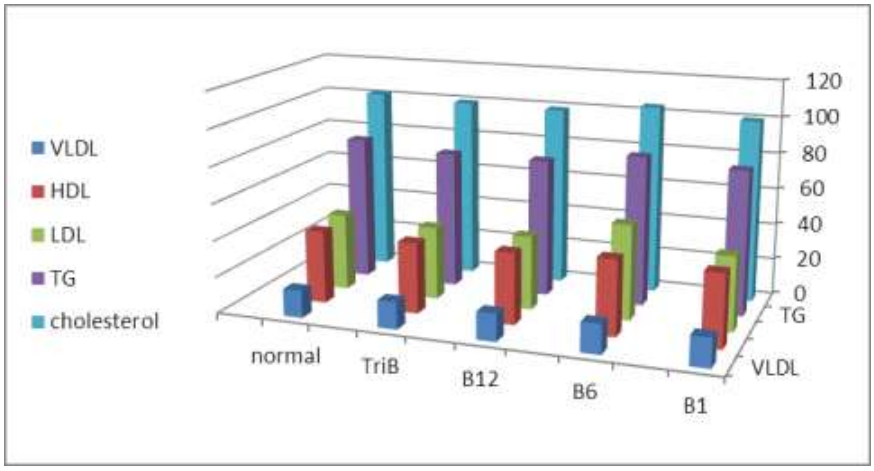

Fig. 1: Histogram showing lipid profile levels among experimental groups and control.

Table1: Lipid profile levels among experimental groups and control.

Lipid Vit.	Cholesterol	TG	LDL	VLDL
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
B1	100±5.477	79.2±7.293	40.8±8.366	15.8±4.494
B6	104±2.915	82.2±8.729	52.1±5.572	16.2±4.658
B12	99±4.472	75.8±9.121	40.4±7.127	15.1±6.024
TriB	99.6±4.416	75.6±7.956	40.3±8.820	15±2.549
Normal	101.4±8.633	79.6±6.693	42.6±6.804	15±1.581

Morphometric analysis

Morphometric data of the therapeutic injured sciatic nerves groups according to the types (B1, B6 and B12) and periods of therapy, there was a significant differences ($p = 0.0437$) with only vitamins B12 among therapeutic groups as shown in table-1. When the groups were compared according to the period, a significant differences were found between the 15thand 45th days ($p=0.05$) in all groups. There was no significant differences between 30th and 45th days in all therapeutic groups. From fig. 2, this study was found only the B12 and TriB

groups were significantly differences with other therapeutic groups, while all therapeutic groups shows a significant differences with control group.

Table 2: Morphometric measurement of myelin thickness among therapeutic groups according to different periods.

		Groups					
Day	No	B1/ (µm)		B6/ (µm)		B12/ (µm)	
		Mean	±SEM	Mean	±SEM	Mean	±SEM
15	5	11.72	2.32	10.49	1.08	14.95	1.22
30	5	13.62	2.92	12.95	1.05	16.91	1.46
45	5	18.91	1.96	17.16	0.30	24.02*	1.67

* A significant differences (P = 0.0437) of the sciatic nerve myelin thickness between (30th & 45th days) in all therapeutic groups. Data showed as mean ±STD.

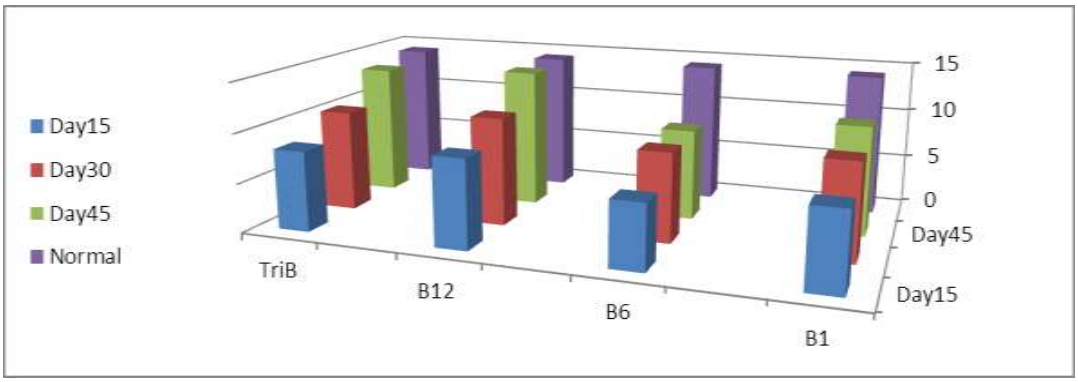

Fig. 2: Showing different period of therapeutic groups with normal control group.

Histological evaluation of the sections

A routine histological technique was applied to all biopsies. Microscopic examination of the control group, which were not submitted to crush, shows a well-organized myelin sheets fibers with a normal orientation of associated fibers fig.3a. On 15th days, there was a diffuse degeneration of the myelinated fibers with a marked vacuolization of some nerve fiber, numerous atrophic disoriented fibers with a large number of mononuclear phagocytes infiltration and degenerative debris fig.3b. Thirty days group shows no vacuolization of nerve sheath and fewer atrophic fibers with few mononuclear and lymphocytes with little degenerative debris. In this group, remyelination of the new fibers was more prominent fig.3c. On 45th days group, there is a clear normal orientation of collagen fiber with very little debris and no vacuolization of the nerve, besides there are no phagocytic cells shown in regenerated nerve fibers. Regeneration fibers were clearly observed and were more prominent than the 30th day fig.3d.

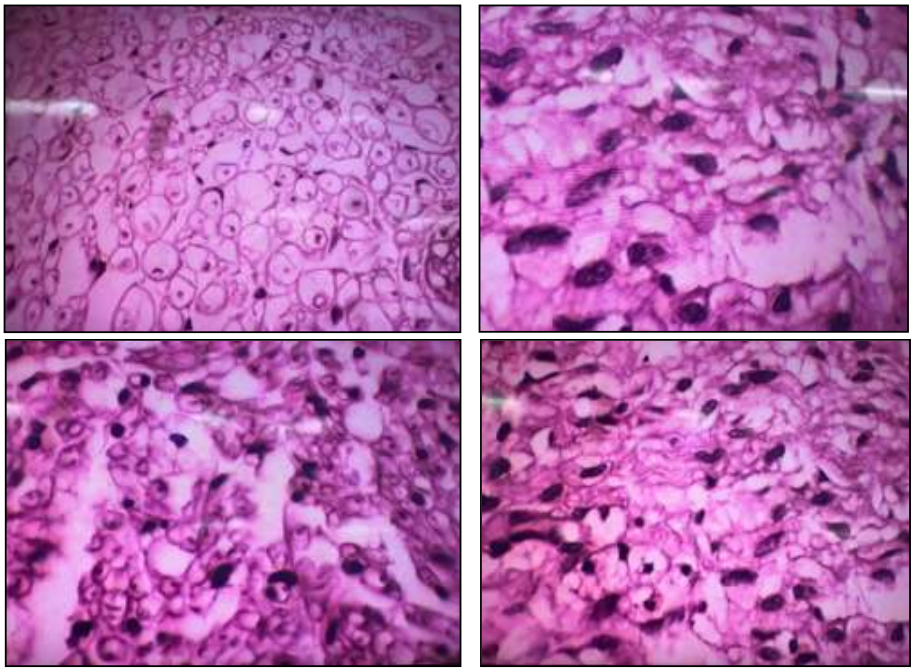

Fig. 3: Histological evaluation of experimental groups. Scale bar 50 μm. (a) Control group, (b) 15th days, (c) 30th days, (d) 45th days of B12 therapeutic group.

DISCUSSION

Experimental design evaluation

The sciatic nerve crush injury model remains the most used and reliable experimental approach in peripheral nerve regeneration studies due to easily assessment functionally and morphologically.[28,29,30] In our study, after crush injury was performed for 60 seconds, a partial functional loss was noticed in the therapeutic groups. A complete clinical recovery was obtained before 30 days post-injury as reported previously with slight differences between 3 to 4 weeks. [31,32,33] This difference may be due to the difference in compression devices during crushing and /or induration of compression, some experiment crush time was very short 20 second. Although the majority of previous studies were taken a shorter time than the experiment we extended at 45th days post-operative even reached clinically normal walking just to study the complete histological repair of the sciatic nerve.

Lipid profile and Vitamin B therapy

Treatment with Tri B during stress is necessary for numerous body processes including metabolism. As reported by the previous studies, a positive association with intakes of certain B vitamins and health was showed.[34,35] The administration of Tri-B into the stressed rats by injection with epinephrine completely improved the changes in TGs, FFAs, total lipids, total cholesterol, LDL, VLDL and HDL after 10 days of repeated treatment.[17,18,36] In addition, vitamin B6 treatment reduced the plasma total cholesterol and LDL-cholesterol in atherosclerotic patients with subnormal plasma B6.[37] The findings of this study further strengthened the previous studies of the role of vitamin B in reducing the peroxidation of fat metabolism and enhancing the catabolism of LDL by inhibiting their glycosylation or stimulation of hepatic cholesterol synthesis and decrease in the activity of the Krebs cycle as reported.[38,39] In our study, the result shows no significant increase in lipid profile during 45 days of the experiment between the control group and the treated group. Although the process of regeneration required more lipid in situ to form new myelin sheath of Schwann cells.[40] Many previous studies show the effect of vitamins B in acceleration rate of lesion peripheral nerves regeneration due to reduced levels of oxidant factors mainly of lipid metabolism.[41-43] These findings are agreed with our results there is no change in lipid profile in each period of taking a blood sample and this may be either the crushing causing weak stress and/ or vitamin B therapy reduce the effects of this stress.

Morphometric measurements and histological evaluation

Among the morphometric measurements of myelin sheath thickness, we chose three types of nerves according to the size (small, medium and large) to avoid any pies and taking into account that the kind of section can be oval or irregular was chosen in our study because it is the most reliable and represents the axon caliber in the cross-sections, also to avoid any bias, and this method of measurement based on.[20] Results of present study shows increase in diameter of treated axons in all groups (B1, B6 and B12) especially between 30th days and 45th days, there is a significant difference of B12 group P = 0.0437 between 30th and 45th days of therapy, and this may due to the activity of vitamin B12 as antioxidant factor and act as neuroprotective mechanism by enhancing the myelination processes through promotion of Lecithin production.[44] Our data are in line with the results reported by[13,19]. The histological changes depended on the severity and duration of the crush. The crush of the sciatic nerve results in either delayed blood flow in the epineurium or complete circulatory arrest according to the intensity and duration of the crush. [45] The crushing intensity of sciatic nerve in our experiment is moderate and takes 60 seconds, but because the covering connective tissue around the sciatic nerve makes the effect of some nerve fibers less affected by crushing. The histological parameters on 15th days of all treated experimental groups samples with vitamins B1, B6, B12 showed moderate degenerative changes (Wallerian degeneration) and the presence of numerous atrophic disoriented fibers, edema with a large number of mononuclear phagocytic cells infiltrated with lymphocytes and increased vacuolization of nerve sheath, these results are assisted other previous studies.[9,21,46] On 30th days of therapy, the affected area shows a presence of more regenerated and less degenerated myelinated nerve fibers, besides the presence of few mononuclear phagocytic cells and lymphocytes, these histological pictures indicate that the regeneration process is still not completed and the myelination process being unfinished. Between the therapeutic group, vitamin B12 group is the best, shows a high degree of regenerative improvement, no vacuolization, very less disoriented fibers, and very few mononuclear cells and lymphocytes were shown beside a large number of Schwann cells along regenerated axons. Nevertheless, decrease number of mononuclear phagocytic cells and lymphocytes recruitment lead to decrease in the damage of nerve cells and improve functional recovery. Our result is in line with the results reported. [47-50] .Although all therapeutic rats show clinically healthy at 30th days of injection, we try to extend the period to 45th days to follow the complete histological pictures. On 45th days, all therapeutic groups show a lesser amount of collagen fibers as compared with the normal group and the arrangement of such fibers was in a well-organized form. Very few macrophages may be found in situ to regulate a complete process of regeneration. Perineurium with a thicker band of fibers while endoneurium with thinner fibers was found. Besides the number and density of the regenerated myelinated fibers were higher and look like a control group. Histological examination of sciatic nerves strongly supported the beneficial effect of vitamin B12 in axonal regeneration due to accelerating the myelination of the nerve beside activating the neurons for production of the neurotransmitter substances as compared to the control group. Our result confirms the previous studies that have indicated that vitamin B12 plays an essential role in the process of neuronal regeneration through myelination of the nerve injured repair. [21,51,52] . Thus from this study, it was concluded that there are no changes of lipid profile level in rat experimentally crushed the sciatic nerves treated with vitamins B. In addition, we confirm that the vitamins B12 is better than other vitamins B, it promotes the acceleration of peripheral nerve regeneration.

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