



# Vitamin B complex and vitamin B<sub>12</sub> levels after peripheral nerve injury

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## Abstract

The aim of the present study was to evaluate whether tissue levels of vitamin B complex and vitamin B<sub>12</sub> were altered after crush-induced peripheral nerve injury in an experimental rat model. A total of 80 male Wistar rats were randomized into one control ( $n = 8$ ) and six study groups (1, 6, 12, 24 hours, 3, and 7 days after experimental nerve injury;  $n = 12$  for each group). Crush-induced peripheral nerve injury was performed on the sciatic nerves of rats in six study groups. Tissue samples from the sites of peripheral nerve injury were obtained at 1, 6, 12, 24 hours, 3 and 7 days after experimental nerve injury. Enzyme-linked immunosorbent assay results showed that tissue levels of vitamin B complex and vitamin B<sub>12</sub> in the injured sciatic nerve were significantly greater at 1 and 12 hours after experimental nerve injury, while they were significantly lower at 7 days than in control group. Tissue level of vitamin B<sub>12</sub> in the injured sciatic nerve was significantly lower at 1, 6, 12 and 24 hours than in the control group. These results suggest that tissue levels of vitamin B complex and vitamin B<sub>12</sub> vary with progression of crush-induced peripheral nerve injury, and supplementation of these vitamins in the acute period may be beneficial for acceleration of nerve regeneration.

**Key Words:** nerve regeneration; sciatic nerve injury; vitamin B complex; vitamin B<sub>12</sub>; neural regeneration

## Introduction

Following peripheral nerve injury, a complex reparative process starts to eliminate the damage and restore the structure and function. Unlike cellular repair in other areas in human body, peripheral nerve injury does not involve mitosis and cellular proliferation (Burnett and Zager, 2004). With advances in molecular biology, increasing attention has been paid to the process of peripheral nerve regeneration. The response of peripheral nerve to injury extends beyond the site of damage but involves neural cells in the spinal cord and ganglia. Schwann cells, macrophages, inflammatory cells, and neurotrophic factors are involved in this complex cascade (Rishal and Fainzilber, 2010). The injured peripheral nerve attempts to compensate the lost functions by strengthening and reprogramming the uninjured pathways (Burnett and Zager, 2004). The whole regeneration and repair process after peripheral nerve injury has not been completely understood. The peripheral nerves are capable of regeneration after injury on their own due to the activation of their intrinsic growth capacity (Rishal and Fainzilber, 2010). The sciatic nerve is commonly used for studying peripheral nerve regeneration since it consists of both sensory and motor neurons (Bridge et al., 1994; Hobbenaghi et al., 2012).

Vitamin B complex helps to alleviate degeneration in the nervous system and vitamin B<sub>1</sub> (thiamine), vitamin B<sub>6</sub> (pyridoxine) in combination with vitamin B<sub>12</sub> are clinically administered (Corinne et al., 2009). These vitamins, in particular vitamin B<sub>12</sub>, exhibit important roles in various biological events to maintain normal neural functions (Corinne

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et al., 2009; Hobbenaghi et al., 2012). Application of vitamin B complex or vitamin B<sub>12</sub> has been shown to increase the number of Schwann cells and myelinated nerve fibers and the diameter of axons, and thereby promote the regeneration of myelinated nerve fibers and the proliferation of Schwann cells (Lopatina et al., 2011). Treatment with vitamin B complex or vitamin B<sub>12</sub> increases the expression of brain-derived neurotrophic factor (BDNF) in the injured sciatic nerves at both mRNA and protein levels, but it does not affect on the expression of glial cell-derived neurotrophic factor, neurotrophin-3, and interleukin-6; in addition, vitamin B complex and vitamin B<sub>12</sub> have been shown to promote the regeneration and functional recovery of injured sciatic nerves through increasing BDNF expression (Sun et al., 2012). Supplementation of vitamin B<sub>12</sub> may enhance peripheral nerve regeneration (Watanabe, 1994). High dose vitamin B<sub>12</sub> has been shown to have the potential to treat peripheral nerve injury (Okada and Tanaka, 2010).

The aim of the current study was to evaluate whether vitamin B complex and vitamin B<sub>12</sub> levels were affected after crush-induced peripheral nerve injury in an experimental rat model. To the best of our knowledge, no articles have been published on this topic and our study is the first to investigate the levels of vitamin B complex in injured peripheral nerve tissue in animal models.

## Materials and Methods

### Animals

This study was approved by Institutional Animal Care and

Use Committee of Kahramanmaraş Sutcu Imam University Medical Faculty. Eighty Wistar rats obtained from Experimental Animal Room of Kahramanmaraş Sutcu Imam University Medical Faculty were included in this study. They were kept at constant temperature (20–22°C) and humidity (50–60%) in 12-hour dark/light cycle and given with *ad libitum* food and water. Procedures were carried out in accordance with the guidelines of the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). All efforts were made to minimize the number of animals used and their suffering.

### Surgical procedures

Eighty Wistar rats, weighing 200–250 g were randomly divided into one control group ( $n = 8$ ) and six study (1<sup>st</sup>, 6<sup>th</sup>, 12<sup>th</sup> hours, 3<sup>rd</sup>, 7<sup>th</sup> days) groups after experimental nerve injury;  $n = 12$  for each group). Surgical interventions were carried out under anesthesia with a premixed solution containing 15 mg/kg xylazine and 100 mg/kg ketamine. Rat models of peripheral nerve injury were established as described by Renno et al. (2013). The right sciatic nerve was exposed at the mid-thigh level and crushed at the location 1 cm from the sciatic notch for 60 seconds with a mosquito forceps. The skin wound was closed with fine sutures. In the control group, the right sciatic nerves identifiably underwent anesthesia and the incisions were closed without any surgical intervention on sciatic nerve. In the control group, immediately after the sciatic nerve was exposed, sciatic nerve tissue was harvested for biopsy. All procedures were implemented by the same surgeon (Idiris Altun) using the same equipment. Nerve injury may depend on the length of time of crush insult. During our earlier trials, we tried different time frames ranging from 10 to 120 seconds. The 60 second compression injury in sciatic nerve showed all the motor and sensory symptoms of a typical crushed nerve reported previously by many investigators (Bridge et al., 1994; Sun et al., 2012; Hobbenaghi et al., 2013; Renno et al., 2013). In the current six study groups, sciatic nerve tissues were harvested at different time points (1, 6, 12, 12 hours, 3 and 7 days after crush injury) for biopsies.

### Preparation of nerve tissue homogenates

Nerve tissue samples were homogenized in two volumes (w/v) of the 1.15% ice-cold KCl solution, using a Heidolph 50110 R2R0 homogenizer (Schwabach, Germany). The homogenate was centrifuged in a Sorvall RC-2B (Minneapolis, MN, USA) at  $39,880 \times g$  for 30 minutes at 4°C and the supernatant was collected for biochemical assays.

### ELISA test procedure

Vitamin B<sub>12</sub> levels were determined according to the test procedure of Ridascreen Fast Vitamin B<sub>12</sub> ELISA (R-Biopharm A.G. Kit, Art No: R2102 Darmstadt, Germany) and results were evaluated using an ELISA reader (DAS). A sufficient number of wells were inserted into the microwell holder for all standards and samples to be run. The standard and sample positions

were recorded. Absorbance at 450 nm was measured.

### Detection of vitamin B complex and vitamin B<sub>12</sub> levels

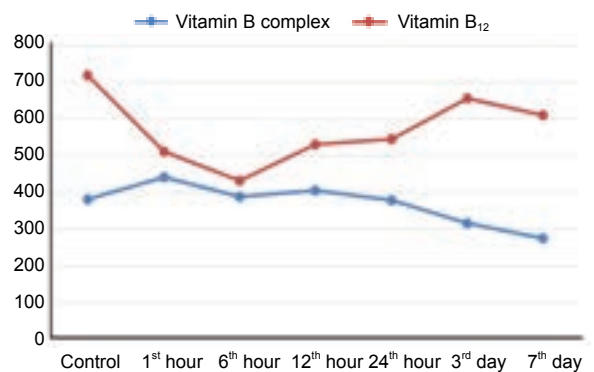
Vitamin B complex and vitamin B<sub>12</sub> levels in tissue homogenate were determined according to the test procedure of Eagle Biosciences Vitamin B<sub>12</sub> ELISA (Catalog Number B1206-K01 Nashua, New Hampshire, USA) and results were evaluated using an ELISA reader. The ELISA assay was performed as above.

### Statistical analysis

Data analysis was performed *via* IBM Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA) and normal distribution of quantitative data was checked with the Kolmogorov-Smirnov test. Parametric tests were applied to normally distributed data, while non-parametric tests were administered for normally distributed data. One-way analysis of variance was used for comparison of groups with independent continuous variables and Duncan's multiple range test was used for comparison tests. Continuous data were expressed as the mean  $\pm$  SD or median [minimum-maximum] and a level of  $P < 0.05$  was considered statistically significant.

### Results

Data collected in this study were found to have a normal distribution ( $P = 0.857$  for vitamin B complex;  $P = 0.926$  for vitamin B<sub>12</sub>). One-way analysis of variance showed that there was significant difference in vitamin B complex level between groups ( $P = 0.001$ ) and homogeneous subsets were designated according to the results of Duncan's multiple range tests (Table 1). Biopsies of sciatic nerve taken at 1, 12 hours and 7 days after experimental nerve injury showed the most significant differences as compared to the control group. Higher level of vitamin B complex was detected in sciatic nerve taken at 1 and 2 hours after experimental nerve and lower level of vitamin B complex in sciatic nerve taken at 7 days ( $P < 0.05$ ). The mean levels of vitamin B complex



**Figure 1** Mean vitamin B complex and vitamin B<sub>12</sub> levels in the injured sciatic nerve of rats.

Compared to the control group, higher level of vitamin B complex was detected in sciatic nerve taken at 1 and 2 hours after injury and lower level of vitamin B<sub>12</sub> in the sciatic nerve taken at 7 days after injury ( $P < 0.05$ ). One-way analysis of variance followed by Duncan's multiple range tests was used for comparisons.

**Table 1** Duncan’s multiple range test results for vitamin B complex levels in study and control groups

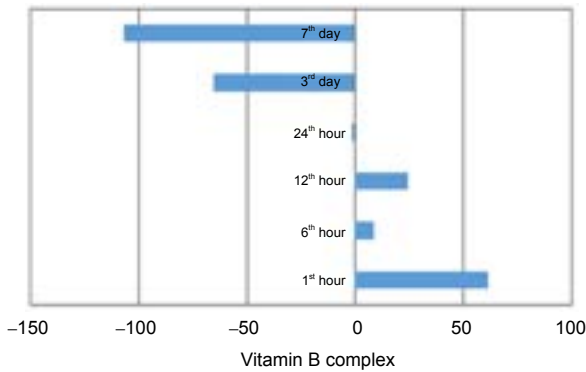
Group	mean±SD	Homogeneous subsets		
		1	2	3
7 <sup>th</sup> day	274.50±80.85	*		
3 <sup>rd</sup> day	315.67±100.70	*	*	
24 <sup>th</sup> hour	379.58±98.44		*	*
Control	381.00±87.51		*	*
6 <sup>th</sup> hour	389.83±115.09		*	*
12 <sup>th</sup> hour	405.58±88.02			*
1 <sup>st</sup> hour	442.50±82.56			*

SD: Standard deviation. \*: Statistically significant.

**Table 2** Duncan’s multiple range test results for vitamin B<sub>12</sub> levels in study and control groups

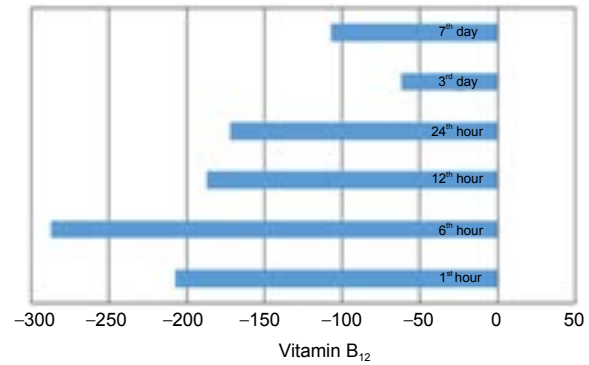
Group	mean±SD	Homogeneous subsets			
		1	2	3	4
1 <sup>st</sup> hour	511.83±98.71	*	*		
6 <sup>th</sup> hour	431.83±94.00	*			
12 <sup>th</sup> hour	531.67±107.95	*	*	*	
24 <sup>th</sup> hour	547.00±175.00	*	*	*	
7 <sup>th</sup> day	611.50±225.46		*	*	*
3 <sup>rd</sup> day	656.83±142.47			*	*
Control	718.75±199.69				*

Significant differences were detected between control and 1<sup>st</sup> hour, 12<sup>th</sup> hour or 7<sup>th</sup> day groups ( $P < 0.05$ ). \*: Statistically significant. SD: Standard deviation.



**Figure 2** Deviation of vitamin B complex level in the study groups from that in the control group.

Data were expressed as the mean ± standard deviation.



**Figure 3** Deviation of vitamin B<sub>12</sub> level in the study groups from that in the control group.

in the control and study groups are shown in **Figure 1**. Deviations of the mean values of the study groups from the control group are shown in **Figures 2** and **3**. One-way analysis of variance results showed that there was significant difference in vitamin B<sub>12</sub> level between groups ( $P = 0.001$ ) and four homogeneous subsets were designated according to the results of Duncan’s multiple range tests (**Table 2**). Significant differences were detected between control and 1<sup>st</sup> hour, 12<sup>th</sup> hour and 7<sup>th</sup> day groups. In 1-hour, 12-hour and 7-day study groups, the mean levels of vitamin B<sub>12</sub> were significantly lower than that in the control group ( $P < 0.05$ ).

### Discussion

In the current study, we aimed to investigate whether vitamin B complex and vitamin B<sub>12</sub> levels in rat sciatic nerve tissue were affected after experimental nerve injury. Our results indicate that peripheral nerve injury is accompanied by various levels of vitamin B complex and vitamin B<sub>12</sub>.

B vitamins such as thiamine (B<sub>1</sub>), pyridoxine (B<sub>6</sub>) and cyanocobalamin (B<sub>12</sub>) have antinociceptive effects in experimental animals with acute and chronic pain linked with neuronal injury (Wang et al., 2005; Caram-Salas et al., 2006; Yu et al., 2014). Vitamins B<sub>6</sub> and B<sub>12</sub> have been shown to counteract the deleterious effects of ischemia on neurons (Kaneda et al., 1997; Hung et al., 2009). These vitamins

play important roles in nutrition, nerve conduction, axonal transport, synthesis of neurotransmitters and excitation *via* cyclic guanosine monophosphate (c-GMP) signaling pathway (Wang et al., 2005). Therefore, reconstructive activity of vitamin B complex on degenerative nerves may facilitate the recovery of nerve function after peripheral nerve injury. Resolution of peripheral nerve injury may benefit from a multinutrient treatment approach since vitamin B complex are necessary for optimum functioning of the nervous system (Maladkar et al., 2014). Unavailability of these vitamins may decrease the enzymatic activity of some enzymes and result in neurotoxicity.

Vitamin B<sub>12</sub> is a scavenger of the reactive oxygen species and has a neuroprotective function owing to its anti-apoptotic and anti-necrotic effects on neurons (Sun et al., 2012; Liao et al., 2013). Vitamin B<sub>12</sub> increases the regeneration of axons and the metabolic pathway of vitamin B<sub>12</sub> is closely related to neuronal survival and repair after injury (Dominguez et al., 2012; Sun et al., 2012; Hobbenaghi et al., 2013). Improvement of the delivery system for B vitamins may provide both new eras for treatment of nerve injuries and yield new insights for understanding the pathophysiology. These vitamins may have synergistic effects that cause production of endogenous neurotropic factors, which enhance peripheral nerve repair (Sun et al., 2012).

Hopefully, investigations into this area will undoubtedly yield information that will lead to surgical and therapeutic advances for treatment of peripheral nerve injury such as the use of neurotrophic factors to stimulate growth and direct growing axons to their proper target organ. Perhaps this additional understanding will also help explain why the central nervous system has not developed such a restorative process and illuminate some possible strategies for simulating the peripheral nerve regenerative microenvironment in the brain and spinal cord. Efforts must be directed for prevention of deficiency of these vitamins and the value of vitamin B supplementation in cases of peripheral nerve injury needs to be investigated in further trials.

The limitations of the current study include lack of histopathological examination and disadvantages of experimental design. Moreover, biochemical results are prone to be influenced by technical and environmental factors. Due to these factors, associations must be made with caution.

To conclude, we demonstrated that tissue levels of vitamin B complex and vitamin B<sub>12</sub> vary with progression of peripheral nerve injury. Supplementation of vitamin B complex in the acute period of peripheral nerve injury deserves to be considered as a treatment option, which may be useful for acceleration of nerve regeneration.

**Author contributions:** *IA designed the study, performed surgery and wrote the paper. EBK provided assistance in technique application. Both of these two authors approved the final version of the paper.*

**Conflicts of interest:** *None declared.*

**Plagiarism check:** *This paper was screened twice using Cross-Check to verify originality before publication.*

**Peer review:** *This paper was double-blinded and stringently reviewed by international expert reviewers.*

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