

Electrophysiological profile of serum vitamin B12 levels, correlation with serum methylmalonic acid levels, and determination of subclinical peripheral nerve involvement

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Ethics Committee Approval

This study was approved by the Ethics Committee of Kirikkale University (date: 01.10.2018, App Nb:2018/15 Project No: 15/01).

All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest

No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Vitamin B12 is essential for normal neural conduction in peripheral nerves. This study aimed to investigate the electrophysiological profile for varying degrees of serum B12 levels and to determine whether a correlation existed between electrophysiological profiles and serum methylmalonic acid (MMA) levels. Also, determination of subclinical peripheral nerve involvement with cold administration was planned in serum B12 levels.

Methods: A total of 101 (63 females, 38 males) subjects with known serum vitamin B12 levels were selected randomly from the neurology outpatient clinic for this study. The subjects were divided into three groups based on the serum total Vitamin B12 levels: (1) B12 deficiency (<126 pg/mL), (2) low B12 (126-250 pg/mL), and (3) normal B12 (250-500 pg/mL). Serum MMA and nerve conduction studies (NCS) were assessed and compared between the three groups. After the cooling procedure was applied to the ulnar and sural nerves, NCS was repeated.

Results: There were 13 subjects in the B12 deficiency group, 44 subjects in the low B12 group, and 44 subjects in the normal group. We found that ulnar sensory nerve action potential amplitudes were significantly decreased ($P = 0.009$), ulnar F latency ($P = 0.003$; $P < 0.001$) was prolonged, and peroneal combined muscle action potential amplitudes decreased ($P = 0.026$) in the B12 deficiency when compared with the low and normal B12 groups. Sural nerve amplitude and conduction velocities were found to be significantly abnormal after the cold application in all groups ($P < 0.001$). The increase in sural nerve sensory nerve amplitude potentials (SNAP) amplitudes was higher in the B12 deficiency group than in the other groups. Mean serum MMA levels were high in all groups. A correlation of nerve conduction study (NCS) changes with serum vitamin B12 and MMA was not observed in the groups after cold application.

Conclusion: Vitamin B12 deficiency may cause subclinical sensorial and motor axonal nerve conduction changes. Nerve conduction changes may not always reach pathological values based on electrophysiological studies but may be detected after cooling administration even in the normal serum B12 levels. A correlation between serum MMA and vitamin B12 levels was found. Therefore, serum levels of vitamin B12, which is important for nerve conduction, should be carefully evaluated in clinical practice.

Keywords: Vitamin B12, Neuropathy, Cold, Biomarkers

Introduction

Vitamin B12 (cobalamin) is a water-soluble vitamin that has a role in cellular and mitochondrial metabolism. The most common reason for its deficiency is inadequate dietary vitamin B12 intake in adults and malabsorption in the elderly [1]. In the laboratory, it is usually diagnosed through measurements of serum vitamin B12 levels. Although no agreed-upon cut-off points for B12 deficiency and low serum B12 levels have been established, values of 148 pg/mL for B12 deficiency and 260 pg/mL for low B12 levels are frequently used [2]. Holotranscobalamin (holoTC), homocysteine, and methylmalonic acid (MMA) are other markers of vitamin B12 deficiency [3]. The occurrence of the typical neurological symptoms may be expected when serum vitamin B12 levels are generally lower than 200 pg/mL, but they may also occur at levels up to 400 pg/mL [4]. Symptoms and serum vitamin B12 levels may not be correlated, and neurological manifestations might be seen before hematological disorders are detected [5].

Vitamin B12 acts as a coenzyme in the synthesis of myelin and plays a role also in the conversion of methylmalonyl-CoA into succinyl-CoA in the nervous system. In cases of vitamin B12 deficiency, this reaction is insufficient and leads to elevated MMA and impairment in the structure of myelin. The methionine synthase reaction in DNA synthesis is also impaired, which leads to elevated levels of homocysteine [6]. Vitamin B12 also has a neuroprotective effect via the action of reactive oxygen species, thereby causing an increase in axonal regeneration and facilitating the repair of neuronal damage [7]. Peripheral neuropathy may be seen in approximately 25% of subjects with B12 deficiency that may be associated with various pathologies. Neuropathy is mostly axonal. Symptoms are usually symmetrical, painless, and sensorial. Some subjects may also have subclinical involvement based on electrophysiological studies [8].

The effects of temperature were examined using electrophysiological studies based on conduction physiology in peripheral nerves, a technique that could be used to reveal some conduction pathologies or clinical findings [9]. The cooling procedure might reveal subclinical peripheral nerve involvement that is associated with the effects of different serum B12 levels on peripheral nerve conduction.

The aims of our study were to investigate the electrophysiological profiles for varying degrees of serum B12 levels ranging from deficient/low to normal and to determine whether a correlation exists between electrophysiological profiles and serum MMA levels, which is known as a functional marker of B12 deficiency. Also, determination of subclinical peripheral nerve involvement after cold administration was planned in serum B12 levels in this study.

Materials and methods

Study design and subjects

Subjects who presented to the neurology outpatient clinic to nonspecific headache and dizziness without neuropathic symptoms and known serum vitamin B12 levels were enrolled and categorized into three groups based on serum vitamin B12 levels. First, the subjects were separated into those with B12

deficiency (<126 pg/mL) and those with serum B12 levels within laboratory range (reference range in our laboratory: 126–500 pg/mL). Subjects who had serum B12 levels within the laboratory range were further divided into two groups: (1) low B12 (126–250 pg/mL) and (2) normal B12 (250–500 pg/mL). Venous blood samples were taken from three groups to measurement MMA. Electrophysiological studies and neurological examinations were performed by a neurologist. Results of nerve conduction studies (NCS) were compared between the three groups by the same neurologist.

Exclusion criteria for subjects consisted of plexopathy, radiculopathy, diabetes mellitus, acute/chronic renal dysfunction, thyroid disease, metabolic disorders, malignancy, history of chemotherapy and/or radiotherapy, vasculitis, alcohol/substance addiction, neurodegenerative diseases, presence of diagnosis of polyneuropathy, and/or symptoms suggesting neuropathic pains.

This study was approved by the institutional ethics committee (15/01-01.10.2018). Informed consent was waived from all subjects before the study.

Electrodiagnostic methods and cooling

NCS were performed on a Medelec Synergy electromyography (EMG) device at room temperature (25 °C) in the neurophysiology laboratory of a tertiary center. The skin and electrode felts were cleaned before the measurements were taken to minimize skin resistance. In each subject, NCS of the median (right), ulnar (left), tibial (right), peroneal (left), and sural (left) nerves were performed using superficial bipolar electrodes. The amplitude and distal motor latency (DML) of compound muscle action potentials (CMAP) and nerve conduction velocities (NCV) were measured after stimulation of the median nerve (wrist/elbow), ulnar nerve (wrist, lower and upper elbow), common peroneal nerve (ankle/fibula head), and tibial nerve (ankle/knee) after supramaximal stimulation in motor conduction studies. The amplitude of the sensorial nerve action potential (SNAP) and sensorial NCV were evaluated using orthodromic methods in the median nerve (second finger interphalangeal joint space and palm), ulnar nerve (fifth finger- wrist with a distance of 12 cm), and sural nerve (lateral malleolus-foreleg with a distance of 14 cm) conduction studies. F-wave latency of each motor nerve was evaluated. Amplitude measurements were calculated from baseline to negative peak, and sensory and motor distal latency measurements were also calculated from baseline to the first negative peak. The laboratory reference values were considered a sural sensorial nerve velocity of > 40 m/s and amplitude > of 5 μ V [10].

Cooling was carried out at 18 °C with a freezing air-free ice pack on the skin over the ulnar nerve and sural nerve tract for 10 min after which skin temperature (< 25 °C) was confirmed with a superficial heat meter of an electromyography (EMG) device. It was confirmed that the EMG device was cooled to lower 25 °C. Ulnar nerve motor conduction, F-wave latency, and sural nerve conduction studies were repeated after cold application.

Measurement of serum methylmalonic acid (MMA)

Serum MMA levels were measured in venous blood samples using micro enzyme-linked immunosorbent assay (ELISA) Andy Gene Biotechnology Co. Ltd, Serial number: AD20022Hu). Biochemical tubes without preservatives were

filled with 5 mL of blood, centrifuged for 30 min at 2000 rpm, and stored at -80°C until used for analysis.

All samples were studied concurrently. The measurement range of the reagent was 0.5 to 40 pg/mL. Samples were studied by making 5-fold dilutions with sample diluents. Samples over 200 pg/mL were studied with 10-fold dilutions. The sensitivity of the reagent was 0.1 pg/mL. The results of test were expressed in nmol/L (laboratory range: 0.05–0.26 nmol/L, cutoff point for diagnosing functional cobalamin deficiency was taken as 0.376 nmol/L) [11].

Statistical analysis

Based on the research of Leishear and colleagues, sample number was calculated with G.Power3.0.10. This study was designed as cross-sectional in a similar way with his study as a reference. Leishear and his colleagues carried out a cross-sectional research study on B12 vitamin levels and peripheral nerve function involving 2287 participants. In this study, the vitamin B12 deficiency was found in 0.069% of the participants and an additional 10.1% had low serum B12 levels. Also, in this research, B12 deficiency was associated with worse sensory and motor peripheral nerve function (odds ratio [OR]: 1.50; 95% confidence interval [CI] 1.06–2.13). With the resulting data, the sample that was calculated with α err prob = 0.05, Power ($1-\beta$ err prob) = 0.80 was found to be 101.

Study data were evaluated using the SPSS 22.0 statistical package program. Descriptive statistics were expressed as means (standard deviations), medians (min–max), frequency distributions, and percentages. For categorical variables, the difference between the groups in terms of frequency was evaluated using a chi-squared test. The suitability of continuous variables to normal distribution was evaluated using the Kolmogorov–Smirnov or Shapiro–Wilk test. Numerical variables showing normal distribution among the groups were evaluated using one-way analysis of variance (ANOVA), and non-normally distributed numerical variables using the Kruskal–Wallis test. If a statistically significant difference was found, the origin of the difference was determined using post hoc tests (Bonferroni test/Mann–Whitney U test). The evaluation of conduction changes before and after cold application was performed using the paired t-test for data with normal distribution and Wilcoxon's signed-ranks test for data without normal distribution. The relationship between vitamin B12 and MMA levels was evaluated using Spearman's correlation test. Statistical significance was accepted as $P < 0.05$.

Results

A total of 101 (63 females, 38 males) subjects with a mean age of 30.8 (10.24) years were included in the study. Thirteen subjects were included in the B12 deficiency group (serum levels <126 pg/mL), 44 subjects in the low B12 group (serum levels: 126–250 pg/mL), and 44 subjects in the normal group (serum levels: 250–500 pg/mL). Adverse effects were not observed due to the cold application.

The characteristics of the groups are shown in Table 1.

Table 1: Characteristics of groups

Data	B12 deficiency (n = 13)	Low B12 (n = 44)	Normal B12 (n = 44)	P-value
Age, years	32.69 (14.38)	28.72 (9.27)	32.31 (9.62)	0.191
Gender, female/male, n	2/11	29/15	32/12	0.001 ²
Vitamin B12 (pg/mL)	104.91 (12.26)	200.22 (37.94)	335.71 (60.90)	$<0.001^1$
Serum MMA level (nmol/L)	148.84 (230.4)	88.79 (103.4)	84.68 (125.6)	0.952

Data shown are mean (SD) unless otherwise specified. ¹ Kruskal–Wallis test, ² Chi-squared test

Results of nerve conduction studies

Motor nerve conductions

Motor nerve conduction values were found to be normal according to age and length in all groups. However, we found statistically significant differences between the groups.

In the upper extremities, ulnar nerve F latency was found to be significantly between the groups ($P = 0.001$). It was prolonged only in the low B12 group (mean 28.01 [1.8] ms in B12 deficiency, 26.51 (1.64) ms in low B12, and 26.10 [1.41] ms in normal B12, $P = 0.001$). The statistical differences were significant between the B12 deficiency and low B12 ($P = 0.003$) and the B12 deficiency and normal B12 ($P < 0.001$) groups. Other parameters, such as compared motor action potential and distal motor latency (CMAP and DML, respectively) for ulnar and median nerve motor conduction studies did not show statistically significant differences.

Tibial nerve DMLs were found to be different in the groups ($P = 0.026$). DMLs for tibial nerve were found to be significantly prolonged in the B12 deficiency and low B12 groups when compared with the normal B12 (4.58 [0.50] ms in B12 deficiency, 4.49 [0.50] ms in low B12, and 4.26 [0.43] ms in normal B12; $P = 0.024$ and $P = 0.038$, respectively). Other parameters for peroneal and tibial nerve motor conduction studies showed no statistically significant differences in the lower extremities.

Sensorial nerve conductions

Ulnar (digit V) SNAP amplitudes were found to be significantly lower in the B12 deficiency versus the low B12 and normal B12 groups (12.53 [3.02] μV in B12 deficiency, 24.95 [2.3] μV in Low B12, and 25.02 [14.74] μV in normal B12; $P = 0.009$). No statistically significant differences between the groups according to sural nerve sensorial conduction studies were detected ($P > 0.05$).

Cooling procedure

In all groups, no significant differences in CMAP amplitudes (distal) and NCV (proximal) parameters of the ulnar nerve (before and after cold) were found ($P > 0.05$). However, DML and F-wave latency led to a significant prolongation and NCV (distal) was significantly decreased in the ulnar nerve after cold application ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively). CMAP amplitude (proximal) significantly decreased only in the low B12 group.

For the sural nerve, significant increases in SNAP amplitude and a decrease in NCV in all groups were found ($P < 0.001$). The increase in SNAP amplitude of the sural nerve was higher in the B12 deficiency group than in the other groups (B12 deficiency group versus low B12 group; $P = 0.049$, B12 deficiency group versus normal B12 group; $P = 0.154$, and low B12 group versus normal B12 group 3; $P = 0.478$). Although the mean sural NCV of all groups was found to be <40 m/s, no significant differences among the groups in terms of sural NCV ($P > 0.05$) were detected.

Table 2: Pre- and post-cooling nerve conduction parameters of groups

Parameter	B12 deficiency (n = 13)			Low B12 (n = 44)			Normal B12 (n = 44)		
	Pre-cooling	Post-cooling	P-value	Pre-cooling	Post-cooling	P-value	Pre-cooling	Post-cooling	P-value
L, Sural nerve									
SNAP amplitude (μ V)	19.88 (9.31) (5-40.3)	26.26 (12.91) (7.8-54.2)	0.003 ^{1*}	17.25 (8.36) (5-43.3)	19.75 (7.71) (5.6-39.7)	0.004 ¹	17.17 (7.6)	20.83 (8.17)	0.001*
NCV (m/sn)	50.39 (7.72) (37.5-67.6)	33.59 (7.90) (25-52.1)	0.001 ^{2*}	50.82 (5.17) (40-65.3)	32.24 (6.1) (19.7-48)	<0.001 ²	49.74 (4.63)	32.99 (5.6)	<0.001 ²
L, Ulnar nerve									
DML (ms)	2.5 (0.51) (1.88-4.01)	2.65 (0.55) (2.08-4.27)	0.007 ²	2.47 (0.32) (1.93-3.28)	2.7 (0.42) (1.98-3.65)	<0.001 ²	2.55 (0.34)	2.78 (0.41)	<0.001 ²
CMAP amplitude									
Distal (μ V)	11.59 (2.07) (8.2-15.4)	11.28 (2.24) (7.9-16)	0.16 ²	13.04 (1.62) (10.3-17.5)	12.92 (1.96) (9.2-18.5)	0.87 ²	12.77 (2.36)	12.8 (2.44)	0.73
Proximal (μ V)	10.94 (2.18) (7.3-15)	10.26 (2.5) (6.2-15.9)	0.05 ¹	12.3 (1.73) (9.8-17.8)	11.82 (2.06) (7.8-18.2)	0.01 ¹	11.88 (2.33)	11.58 (2.26)	0.08
F wave latency (ms)	28.01 (1.81) (25.16-32.03)	29.84 (2.42) (25.31-33.34)	0.002 ^{2*}	26.51 (1.64) (23.49-29.9)	28.13 (2.19) (24.48-33.18)	<0.001 ²	26.10 (1.41)	27.66 (1.95)	<0.001 ²
NCV, Distal (m/sn)	54.47 (3.83) (47.5-61.8)	48.32 (3.93) (43.7-56.4)	0.002 ^{2*}	56.77 (4.08) (50.3-69.8)	51.12 (5.27) (37.9-64.5)	<0.001 ²	56.75 (40.17)	51.35 (4.87)	<0.001

SNAP: Sensorial nerve action potential, NCV: Nerve conduction velocity, DML: Distal motor latency, CMAP: Compound muscle action potential, Data as mean (SD) or median (min-max) in B12 deficiency and Low B12, mean (SD) in Normal B12, ¹ Paired T test, ² Wilcoxon Signed Ranks test

The pre- and post-cooling nerve conduction parameters of the groups are shown in Table 2.

Correlation between MMA and vitamin B12 levels

Mean MMA levels were high in all groups: (1) 17.56 (27.18) nmol/L (min-max: 0.37-93.83) in the B12 deficiency group, 10.47 (12.20) nmol/L (min-max: 1.55-58.41) in the low B12 group, and 9.99 (14.82) nmol/L (min-max: 0.35-75.24) in the normal B12 group. However, no significant correlation between MMA and vitamin B12 levels between the groups was found. These results are presented for the three groups: (1) B12 deficiency group ($r = -0.084$; $P = 0.79$); (2) low B12 group ($r = 0.027$; $P = 0.86$); and (3) normal B12 group ($r = -0.017$; $P = 0.91$). No significant correlations between MMA and vitamin B12 in the B12 deficiency group ($r = -0.084$; $P = 0.79$), the low B12 group ($r = 0.027$; $P = 0.86$), and normal B12 group ($r = -0.017$; $P = 0.91$) were found.

Correlation between serum MMA, B12 levels, and alterations in nerve conduction parameters after the cooling procedure

Nerve conduction parameter alterations after the cooling procedure were not found to be correlated with any of the serum levels of vitamin B12 or MMA ($P = 0.89$, $P = 0.734$).

Discussion

Vitamin B12 is one of the neurotropic vitamins and contributes to optimal nerve function in the peripheral nervous system. Deficient or functionally deficient levels of the B12-dependent metabolites despite normal serum vitamin B12 levels may affect nerve conduction, which could result in neuropathy [6]. The findings that could be expected based on the electrophysiological studies are slowing of the conduction velocity of the sensory nerves and/or a decrease in SNAP amplitude in the presence of neuropathy due to B12 deficiency although such changes may or may not be correlated with serum B12 levels [12, 13].

In this study, we did not find significant NCS changes, which would be indicative of peripheral neuropathy, in any vitamin B12 level groups even in those with B12 deficiency. Furthermore, sural nerve conduction parameters, which are important parameters for the diagnosis of peripheral neuropathy, were within normal ranges even in the deficiency group [14]. However, we found that a decrease in sensorial (median and ulnar) nerve amplitude, prolonged ulnar F-wave latencies, and tibial DMLs in addition to a decrease in peroneal CMAP amplitudes in B12 deficiency. With this evidence, we suggest that these findings indicate axonal involvement in sensorial fibers and mixed-type involvement in motor nerve conduction parameters in the B12 deficiency group. These findings were found to be statistically significant when compared with the NCS

of other serum B12 level groups. It has been previously reported that the electrophysiological findings of subjects with asymptomatic/symptomatic vitamin B12 deficiency (mean serum level <200 pg/mL) revealed inconsistent pathological and electrophysiological findings. Neuropathy has not been observed electrophysiologically by researchers in any subjects with B12 deficiency [8, 15, 16]. A comparison of symptomatic subjects with B12 deficiency and healthy controls showed that NCS of asymptomatic subjects with vitamin B12 deficiency were not different from healthy subjects [17]. It is also known that nerve conduction changes do not correlate with vitamin B12 levels. However, we observed that sensory nerves might have a tendency to axonal-type involvement in B12 deficiency although not at a neuropathic level.

In this study, another important finding was that sural NCVs achieved neuropathic ranges in all groups after cooling, but no statistically significant differences in terms of the decrease in sural nerve NCVs between the groups after the cooling procedure were detected. In fact, a linear curve between the decrease in temperature and NCV was found, and it is generally reported that a 1 °C variation in surface temperature causes a reduction in NCV of about 1.5 to 2 m/s [18-21]. However, we observed a more apparent reduction in sural NCVs after the cooling procedure. We suppose that myelin might have had an effect on sensory nerves at serum B12 levels of <500 pg/mL. Previous research has shown that electrophysiologically, the underlying demyelinating pathology results from cold application in clinical carpal tunnel syndrome cases with normal nerve conduction [22]. In vitamin B12 deficiency, axonal-weighted features are generally expected in NCS, but an effect of Schwann cells in the peripheral nervous system, which has a closer relationship with axons than oligodendrocytes in the central nervous system, has been shown in animal experiments. Accordingly, Schwann cell activation and intra-myelin edema has been observed to develop without demyelination or remyelination with little or no axonal change [23-25]. After cooling, we also observed a significant increase in sural SNAP amplitudes in all three groups. A remarkable increase in the B12 deficiency group was observed. The effect of temperature on neuromuscular electrophysiology has been reported as an increase in amplitudes and decrease in NCVs, and the change in amplitude is much greater than that seen in normal subjects with axonal disorders [9]. We suggest that the B12 deficiency group might have axonal involvement in sensory nerves as we previously concluded. Asymptomatic subjects with B12 deficiency who had normal NCS at the first examination were found to have abnormal NCS findings after cooling, indicating axonal neuropathy.

We chose the sural and ulnar nerves to reveal silent changes in the peripheral nerves after cold treatment in this study. It is known that cold applications administered via various methods (ice pack, ice massage, cold water immersion) have a more complex effect, particularly on the sensory nerves [9]. We also observed the expected physiological effects on ulnar nerve conduction parameters (amplitude, velocity, F-wave latency, and DML) after cold application, but the changes were not pathological.

In clinical practice, investigation of serum levels of vitamin B12 is the most common approach in the assessment of vitamin B12 deficiency. The levels of homocysteine, holoTC, and MMA can also be used in the laboratory although a lack of consensus on the best marker in this regard exists. Harrington et al. reported that vitamin B12 serum levels lacked the necessary sensitivity for indicating actual deficiencies [26]. In previous studies, Nexo et al. [27] and Herrmann et al. [28] reported that holoTC was being more sensitive if creatine levels were within the normal range. Serum MMA levels may be more reliable for assessing vitamin B12 deficiency in conjunction with holoTC, another metabolite, and for investigating MMA in the urine. MMA is biochemically more stable in urine than in serum in which concentrations are 40 times greater than in serum [29]. Sun et al. reported that the urine levels of MMA may be a marker in the assessment of B12 serum levels in polyneuropathy in diabetic patients [30]. We found high serum MMA levels according to our laboratory range, but no relationship between serum MMA and B12 levels and also NCS was found. Serum MMA levels were not found to have a predictive value for electrophysiological results.

Limitations

This study has some limitations. The B12 deficiency group consisted of a small number of subjects, and we did not examine homocysteine levels. The B12 laboratory reference range in our study appears slightly different from the literature, but this difference did not constitute a limitation. A larger study group and urinary MMA levels might be recommended for future research.

Conclusion

In a summary, we found that subjects who had vitamin B12 deficiency have a tendency to develop axonal/demyelinating-type involvement in both sensory and motor nerve fibers. Nerve conduction changes may not always reach pathological values in electrophysiological studies but may be detected after cooling treatment even in those with normal serum B12 levels. Cooling is an easy test to perform. A correlation between serum MMA and vitamin B12 levels was found. Serum levels of vitamin B12, which is important for nerve conduction, should be carefully evaluated in clinical practice.

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