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# Visual and somotosensory evoked potentials in asymptomatic patients with vitamin B12 deficiency

B. GÖKÇE ÇOKAL, H.N. GÜNEŞ, S. KESKIN GÜLER, T.K. YOLDAŞ

Department of Neurology, Ankara Education and Research Hospital, Ankara, Turkey

**Abstract. – OBJECTIVE: Vitamin B12 deficiency may be asymptomatic or present with a wide range of neurological and hematological disorders. Our aim in this study is to evaluate visual (VEP) and somatosensory evoked potential (SEP) parameters in patients with vitamin B12 deficiency who had no clinical evidence of visual impairment or neurological syndrome findings and compare the findings with healthy controls to determine whether there is a correlation between VEP and SEP parameters and serum vitamin B12 levels.**

**PATIENTS AND METHODS: 30 patients (6 females [20%], 24 males [80%]; mean age, 52 years [range 17-80 years]), and 15 healthy subjects with vitamin B12 deficiency (3 females [20%], 12 [80%] male; mean age, 49 years [range 17-78 years]) were included in the study. P100 wave latencies and amplitudes were recorded as VEP parameters, and P40 wave latencies and amplitudes were recorded as tibial SEP parameters.**

**RESULTS: Comparison of VEP and SEP parameters in the patient and control groups revealed significantly prolonged SEP latencies and lower SEP amplitudes in the patient group. VEP latencies did not significantly differ between the patient and the control groups while VEP amplitudes were found to be lower in the patient group than in controls. A significant correlation was obtained between serum vitamin B12 levels and tibial SEP latencies ($r > 0.5$).**

**CONCLUSIONS: These findings suggest that asymptomatic patients with vitamin B12 deficiency may have SEP and VEP abnormalities indicating the subclinical optic nerve and spinal cord involvement.**

*Key Words:*
Vitamin B12, Visual evoked potentials, Somatosensory evoked potentials, Optic nerve, Spinal cord.

## Introduction

Vitamin B12 is an essential water-soluble vitamin and obtained from foods of animal origin. Vitamin B12 is important for DNA synthesis, myelination and normal function of the central nervous system and haematopoiesis[1-3]. There are numerous causes of deficiency. These include low intake of vitamin B12, pernicious anemia, atrophic gastritis, chronic gastritis, surgery (e.g., post-gastrectomy and ileal resection), genetically transcobalamin II deficiency, malabsorption syndromes and drugs (e.g., oral contraceptives, metformin)[4,5].

Patients with vitamin B12 deficiency can be asymptomatic or may present with a wide range of symptoms of hematological and neurological manifestations such as hypersegmented polymorphs, neutropenia, thrombocytopenia, subacute combined degeneration (SCD) of the spinal cord, peripheral neuropathy, optic neuropathy, Alzheimer's disease, depression, delirium, and psychosis[1,6]. The main cause of neurological damage in vitamin B12 deficiency is not clear. It has been hypothesized that it causes inadequate myelin synthesis and consequently leads to dysfunction in central and peripheral nervous system resulting in demyelination and axonal degeneration.

This study is aimed to evaluate the visual evoked potential (VEP) and the somatosensory evoked potential (SEP) by measurements in patients with vitamin B12 deficiency without clinical evidence of visual impairment and neurological syndrome findings. The aim is to determine whether there are any VEP and SEP abnormalities in asymptomatic patients and to determine whether there is a correlation between VEP and SEP parameters and serum vitamin B12 levels.

## Patients and Methods

We recruited patients from the Outpatient Unit in the Department of Neurology, Etlik Ihtisas Education and Research Hospital. This study was approved by the hospital Ethics Committee.

*Corresponding Author:* Burcu Gökçe Çokal, MD; e-mail: gokceburcu@gmail.com

Written informed consent was obtained from all patients and controls. The vitamin B12 deficiency group was composed of 30 patients (6 females [20%], 24 males [80%]; mean age, 52 years [range 17-80years]) and the control group included 15 healthy subjects vitamin B12 deficiency (3 females [20%], 12 [80%] male; mean age, 49 years [range 17-78 years]). Patients with vitamin B12 serum level < 200 pg/ml and with peripheral blood smear showing hypersegmented neutrophils were considered as vitamin B12 deficiency. The laboratory investigations included hemoglobin, hematocrit, platelet, RBC indices, peripheral blood smear, fasting blood sugar, routine biochemistry, thyroid function tests and HIV serology. The levels were within the normal ranges. Cognitive status of the participants was evaluated by the Mini Mental State Examination (MMSE). A detailed clinical history including dietary intake, chronic alcoholism, malabsorption syndrome, gastrointestinal surgery, taking any drug, history of systemic or autoimmune disorders like thyroid dysfunction, rheumatoid arthritis, diabetes mellitus was obtained. Patients and control subjects were evaluated clinically and electrophysiologically to detect any evidence of peripheral neuropathy. Patients and healthy controls with polyneuropathy were excluded from the study. Patients without neurologic complaints, any spinal cord diseases, any chronic diseases or history of intake of any drugs known to cause peripheral neuropathy were included in the study. All subjects underwent a thorough ophthalmologic examination, including tests for visual acuity, color vision, and visual field. Vitamin B12 levels were measured using chemiluminescent immunoassay kits (Abbott Laboratories, Lake Forest, IL, USA). The reference range for vitamin B12 was 200-900 pg/mL. Spinal and brain magnetic resonance imagings (MRI) were performed in all patients and no abnormalities were detected.

### Visual Evoked Potentials

The VEP recordings were performed using an electromyography (EMG) machine (Nihon Kohden, Tokyo, Japan). Each subject was seated comfortably on a chair in a quiet darkened and electrically shielded room at a distance of 100 cm away from the black-and-white, checkerboard-patterned monochrome screen and instructed to fix the gaze on a square at the center of the screen while the unstimulated eye was covered with an eye patch. Standard silver chloride electrodes were used for recording. Electrodes were placed on the scalp (Oz and Fz), and the ground electrode was placed at Cz using electroencephalogram paste according to the International 10-20 system. We kept electrode impedances in each subject less than 5 KΩ. The high and low frequency filter settings were 1-300 Hz. The check size was 60 minutes of arc. The mean luminance of the checkerboard was 50 cd/$m^2$ and contrast between black and white squares was 99%. Sweep duration was 500 ms and sweep speed was 50 ms/s. The rate of pattern reversal was 1 Hz and an average of 100 responses was recorded twice for reproducibility. The recording was monitored closely by a neurology specialist. P100 latencies and amplitudes were measured in both, right and left eye for each subject.

### Posterior Tibial SEP

Posterior tibial SEP recordings were performed bilaterally using an EMG machine (Nihon Kohden, Tokyo, Japan). Electrodes were placed on the scalp (CZ-FZ) according to the 10–20 system. Tibial nerve was stimulated at the ankle, behind the medial malleol with stimulus intensity is high enough to produce a slight visible in the foot fingers muscle twitch. The high and low frequency filter settings were 10-3000 Hz. Sweep duration was 500 ms and sweep speed was 50 ms/s. An average of 100 responses was recorded twice for reproducibility. The latencies and amplitudes of P40 were measured.

### Statistical Analysis

All statistical tests were performed using the Statistical Package for the Social Sciences software (SPSS ver. 20; SPSS Inc., Chicago, IL, USA. To compare the groups, independent sample t-test was used for the variables with normal distribution, and Kolmogorov-Smirnov test was used for the variables with asymmetric distribution. Values of $p < 0.05$ were considered to be statistically significant. Spearman's rank test was used to examine the correlation between VEP and SEP parameters and serum vitamin B12 levels. Values of $r > 0.5$ were considered to be a significant correlation.

## Results

Neurologically asymptomatic 30 patients with vitamin B12 deficiency (6 females, 24 males) and sex- and age-matched healthy subjects (3 fe-

males, 12 males) were examined. Mean age was 52 years (range 17-80 years) in the patient group and 49 years (range 17-78 years) in the control group. The mean vitamin B12 level was 139.3.±48.4 (N: 200-900) in the patient group and 320±118 in the control group. MMSE scores were 30 both in patient and control groups.

Fifteen of the 30 patients were vegetarian. Thirteen patients underwent upper gastrointestinal endoscopy. Atrophic gastritis, pan-gastritis, and antral gastritis were observed in two, two, and nine patients, respectively. Two patients had a history of gastrointestinal surgery.

The P100 wave latency obtained from the right eye of patients with vitamin B12 deficiency was 112.7 ± 18, and that from the right eye of the controls was 106 ± 5.4 ($p$ = 0.239). The P100 wave latency obtained from the left eye of patients with vitamin B12 deficiency was 112.6 ± 17, and that obtained from the left eye of the control group was 106.5 ± 6.2 ($p$ = 0.110). VEP amplitudes obtained from the right eye of patients with vitamin B12 deficiency and the control group were 5.1 ± 2.8 and 7.7 ± 2.3, respectively ($p$ = 0.012). The VEP amplitude obtained from the left eye in patients with vitamin B12 deficiency was 5.7 ± 2.9 and that in the control group was 8.8 ± 2.3 ($p$ = 0.003). Significant differences were observed in the VEP amplitudes of both eyes between the vitamin B12-deficient and control groups. P100 wave amplitudes were lower in the patient group than those in the controls.

In an SEP study, P40 latency of the tibial nerve on the right side in patients with vitamin B12 deficiency was 42.8 ± 5.2 and was 38.1 ± 2.2 in the control group. P40 latency of the posterior tibial nerve on the right side was prolonged in patients with vitamin B12 deficiency compared with that in the control group ($p$ = 0.003). P40 latency of the posterior tibial nerve on the left side in patients with vitamin B12 deficiency was 43.2 ± 5.5 and that in the control was 36.9 ± 2.9. P40 latency on the left side was prolonged in patients with vitamin B12 deficiency compared with that in the control group ($p$ = 0.001). P40 amplitudes of the posterior tibial nerve SEP on the right side were 1.9 ± 1.2 and 3.3 ± 2.2 in the vitamin B12-deficient and control groups, respectively ($p$ = 0.013). P40 amplitudes of the posterior tibial nerve SEP on the left side were 2.0 ± 1.2 and 3.2 ± 2.1 in the vitamin B12-deficient and control groups, respectively ($p$ = 0.023). These results show that P40 amplitudes decreased and that P40 latencies were prolonged in vitamin B12-deficient patients compared with those in the controls. A significant correlation was obtained between serum vitamin B12 levels and tibial SEP latencies ($r > 0.5$).

## Discussion

Vitamin B12 is an essential water-soluble vitamin obtained from animal-sourced foods. Therefore it is common in vegans. It plays an important role in the methylation and the continuation of normal function of the nervous system[7]. Vitamin B12 deficiency patients may be asymptomatic or may present with neurological or hematological abnormalities such as hypersegmented polymorphs, neutropenia, thrombocytopenia, SCD, peripheral neuropathy, optic neuropathy, Alzheimer's disease, depression, delirium and psychosis[1]. The main cause of neurological damage occurring in vitamin B12 deficiency is not clear. It has been hypothesized that vitamin B12 deficiency can cause neurological symptoms by impairing two enzyme systems, leading to inadequate myelin synthesis. Vitamin B12 is a cofactor in two important enzymatic reactions in humans. One hypothesis is that methionine synthesis is affected by vitamin B12 deficiency and that the S-adenosylmethionine level decreases, which is important for producing myelin phospholipids[8].

The most common neurologic manifestations in patients with vitamin B12 deficiency are SCD and polyneuropathy. Optic neuropathy is a rare manifestation of vitamin B12 deficiency[9]. SCD is a demyelinating disease and occurs due to damage of the lateral and dorsal spinal cord as a result of myelin sheath defect. It is manifested by lower limb weakness, paresthesias and numbness, loss of position and vibration sense. Stiffness and spastic ataxia may develop in severely affected patients.

Polyneuropathy in patients with vitamin B12 deficiency is frequently the axonal type[10], and electrophysiological findings have revealed axonal degeneration with or without demyelination[11-14].

Optic neuropathy occurs by demyelination of the white matter in the optic nerve[15]. Focal demyelination of the white matter has been reported in the optic nerve and spinal cord of patients with vitamin B12 deficiency due to prolonged P100 latencies[11,12,16-19] and prolonged somatosensory evoked potential (SEP) latencies[11-14]. In some of these studies, the patients were neuro-

**Table I.** VEP values in patients and controls.

| VEP parameters | Eye tested | Patients (n = 30) mean ± SD | Controls (n = 15) mean ± SD | *p*-value |
|---|---|---|---|---|
| Latency P100 (ms) | Right | 112.7 ± 18 | 106 ± 5.4 | 0.239 |
| | Left | 112.6 ± 17 | 106.5 ± 6.2 | 0.110 |
| Amplitude P100 (μV) | Right | 5.1 ± 2.8 | 7.7 ± 2.3 | 0.012 |
| | Left | 5.7 ± 2.9 | 8.8 ± 2.3 | 0.003 |

VEP = Visual evoked potentials; SD = Standard deviation.

logically symptomatic[11,13,14,19] and, in others, they were neurologically symptomatic but had no visual impairment[12,16-18].

VEP latencies are prolonged in patients with no visual symptoms[12,16-18], suggesting subclinical damage to the optic nerve. The patients in our study had no visual impairment, but no differences were observed in P100 latencies between the patient and control groups according to the VEP latencies. However, the VEP amplitudes were lower than those in the control group.

A search in the literature about visual and somatosensory evoked potentials in neurologically asymptomatic patients with vitamin B12 deficiency resulted in finding two previous studies[20,21]. The results of these studies disagree with our findings. However, the study designs and outcomes were different from those in our work. Domac et al[20] evaluated nerve conduction studies, SEPs, and VEPs in patients with vitamin B12 deficiency without a neurological syndrome. They compared VEPs, tibial and median SEPs, and motor evoked potentials in patient and control groups. They only compared latencies, whereas we compared tibial SEP and VEP latencies and amplitudes in patients with those in healthy subjects. They found no differences in the VEP or SEP latencies between the patient and control groups, which contrasted with our study. Boylu et al[21] investigated polyneuropathy in neurologically asymptomatic patients with vitamin B12 deficiency. Nerve conduction study results and tibial SEP latencies have been evaluated in patients and compared with those in healthy controls. In that report, no difference was observed in tibial SEP latencies between the patient and control groups, which disagree with our results. We observed significant differences in SEP latencies and SEP and VEP amplitudes between patients and healthy subjects. P40 latencies were significantly prolonged, and P40 and P100 amplitudes decreased in the patient group compared with those in the control group.

These findings reveal axonal loss in the optic nerve in asymptomatic patients with vitamin B12 deficiency and show that subclinical spinal cord damage may be present, even though these patients are neurologically asymptomatic.

Furthermore, in our paper, there was a significant correlation between serum vitamin B12 levels and tibial SEP latencies. A few studies evaluated the correlation between vitamin B12 levels and electrophysiological findings. In one of these studies, serum vitamin B12 level was found to correlate with the latencies of tibial SEP[12] as similar to the findings in our report, while in others they have found no correlation between tibial SEP latencies and vitamin B12 levels[13,20,21].

**Table II.** SEP values in patients and controls.

| SEP parameters | Eye tested | Patients (n = 40) mean ± SD | Controls (n = 40) mean ± SD | *p*-value |
|---|---|---|---|---|
| Latency P40 (ms) | Right | 42.8 ± 5.2 | 38.1 ± 2.2 | 0.003 |
| | Left | 43.2 ± 5.5 | 36.9 ± 2.9 | 0.001 |
| Amplitude P40 (μV) | Right | 1.9 ± 1.2 | 3.3 ± 2.2 | 0.013 |
| | Left | 2.0±1..2 | 3.2 ± 2.1 | 0.023 |

SEP = Somatosensory evoked potentials; SD = Standard deviation.

## Conclusions

Our findings suggest that patients with no clinical dysfunction in the visual system or any signs or symptoms related to spinal cord damage may have SEP and VEP abnormalities, indicating the subclinical neurologic involvement and early manifestation of vitamin B12 deficiency. Early diagnosis and management of vitamin B12 deficiency are important to prevent neurologic complications.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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