ABSTRACT

Purpose: To evaluate the peripapillary retinal nerve fiber layer (RNFL) thickness with spectral domain optical coherence tomography (SD-OCT) in pediatric cases who presented with different neurological symptoms and determined with vitamin B12 deficiency.

Materials and Methods: Twenty-six patients diagnosed with vitamin B12 deficiency (study group) and 30 age and sex matched healthy children (control group) were included in this study. Complete blood count, serum vitamin B12 level (pg/mL), serum folic acid level (ng/mL) and complete biochemical parameters were obtained for all subjects in both groups. Peripapillary RNFL thickness measurements were performed with SD-OCT (Optovue Inc. Fremont, CA, USA).

Results: In comparison with the control group values, the superior RNFL and global RNFL thickness measurements of the study group were statistically significantly lower, indicating a thinner layer (p=0.01, p=0.007, respectively). The nasal and inferior RNFL thickness measurements of the study group were lower than those of the control group, however, no statistically significant difference was determined (p=0.069, p=0.097, respectively).

Conclusions: RNFL thickness should be evaluated in children suffer from weakness, dizziness and numbness in the hands and feet and detected low serum vitamin b12 level. Low RNFL values in these patients may be a direct/indirect indicator of central nervous system involvement.

Key words: Retinal nerve fiber layer (RNFL), Optical coherence tomography (OCT), Vitamin B12 deficiency.
the identification of glaucoma patients. Recently, the measurement of RNFL thickness with OCT has been used in the diagnosis of neurological and neuro-ophthalmological diseases such as multiple sclerosis, Alzheimer’s disease and Parkinson’s disease, and clinical axonal damage is determined with thinning of the RNFL.13-15

In literature there are few studies related to the visual pathways in pediatric patients with vitamin B12 deficiency. The goal of the current study was to evaluate the RNFL thickness with Spectral Domain OCT in pediatric cases who presented with different neurological symptoms and were determined with vitamin B12 deficiency in a tertiary level healthcare centre.

MATERIAL AND METHOD

The study included 26 pediatric patients who presented at the Pediatric Neurology Clinic of Kahramanmaras Sutcu Imam University Medical Faculty between 2016 and 2017 with complaints of neurological symptoms such as weakness and fatigue, headache, dizziness, numbness in the hands and feet, convulsions, or fainting and were determined with a vitamin B12 level <180 pg/mL. A control group was formed of 30 age and gender-matched healthy children. Approval for the study was granted by the Ethics Committee of Kahramanmaras Sutcu Imam University. All the study procedures were carried out in compliance with the principles of the Helsinki Declaration. Informed consent was obtained from the parents or legal guardian of all the study subjects.

A detailed clinical evaluation was made of each patient, followed by a detailed neurological examination. Information was obtained in detail about diet history, gastrointestinal disease or any other systemic diseases. Full blood count and biochemical parameters were examined, with vitamin B12 and folate levels defined. The patients included in the study were those with no pathology determined on EEG and cranial magnetic resonance imaging (MRI), no additional neurological or chronic disease, and no abnormality other than evident vitamin B12 deficiency (<180 pg/mL) determined in the laboratory tests, following presentation with various neurological symptoms.

Comprehensive ophthalmological evaluation was made of each patient including best corrected visual acuity (BCVA) (snellen), pupillary reaction, slit lamp biomicroscopy, air puff tonometry, fundus examination after pupil dilatation with 0.5% tropicamide, and SD-OCT measurements. Patients were excluded from the study if they had refractive errors of >2.5 diopters (D) of myopia, 2.5D of hyperopia, and 2 D of astigmatism. Those with a history of ocular trauma or surgery, those with glaucoma, any congenital or acquired retinal disorder, or neuro-ophthalmological disease, were also excluded from the study.

All OCT scans and measurements were acquired by the same experienced operator (A.Ç) using RTVue XR Avanti with AngioVue (Optovue Inc., Fremont, CA, USA). Peripapillary RNFL thickness was measured with the ONH program at a diameter of 3.40 mm around the center of the optic disc. A peripapillary RNFL thickness map was used for the four quadrants: superior, nasal, inferior and temporal. The average RNFL thickness was evaluated using the RNFL thickness analysis table. The data of the right eye of each participant was used in the analysis.

The vitamin B12 and folate levels in the samples were analyzed using an enzyme immunoassay (ELISA) technique.

Data obtained in the study were analysed statistically using the Statistical Package for Social Sciences (SPSS version 17). Descriptive statistics were stated as mean ± standard deviation, minimum- maximum values, number (n) and percentage (%). The Independent Samples t-test was used to analyse quantitative data, and the Chi-square test for qualitative data. A value of p<0.05 was accepted as statistically significant.

RESULTS

Evaluation was made of 26 eyes of 26 patients, comprising 15 (57.7%) males and 11 (42.3%) females with a mean age of 11.69±3.77 years (study group). A control group was formed of 30 age and gender-matched subjects with no systemic or ocular disease, comprising 15 (50%) males and 15 (50%) with a mean age of 10.90±3.49 years. No significant difference was determined between the groups in respect of age and gender (p=0.421, p=0.573, respectively).

The mean vitamin B12 level was determined to be statistically significantly lower in the study group than in the control group (p<0.01). No significant difference was determined between the groups in respect of folic acid levels (p=0.934). No significant difference was determined between the groups in respect of BCVA, intraocular pressure (IOP) (p=0.902), axial length (p=0.739) and spherical equivalent (p=0.094). The mean duration of symptoms in the study group was 6,26±1.82 (3-9) months. The demographic and clinical data of the patients and control group are shown in Table 1.

No abnormality was determined in the hematological and biochemical parameters of the study group cases, and no pathologies were determined on MRI. In the EEG
In vitamin B12 deficiency group, global RNFL and superior RNFL thicknesses were significantly correlated with vitamin B12 level \( (p = 0.027, r = 0.296 \) and \( p = 0.005, r = 0.369 \), respectively). In addition, there was no significant correlation between global RNLF thickness and duration of vitamin B12 deficiency \( (p= 0.197, r = -0.262) \).

**DISCUSSION**

In the present study, we examined the effect of vitamin B12 deficiency in the optic nerve which is a part of the central nervous system in the pediatric age group and we compared the study group with vitamin B12 deficiency to the age and sex-matched healthy group. Since nutritional deficiency of vitamin B12 is the only proven nutritional loss causing optic neuropathy and loss of sight. 6,7 However, the mechanism of this is not fully understood. Vitamin B12 is a co-factor for two enzymes, primarily methionine synthase and methylmalonil-CoA synthase, which convert homocysteine to methionine. Disruption in the conversion of homocysteine to methionine causes defective DNA synthesis and defective production of choline and phospholipid containing choline. 16 These types of lipids are found in myelin sheaths and the damage of cobalamin deficiency may be partly responsible for the optic neuropathy due to vitamin B12 deficiency.17

In a previous experimental study, the pathogenesis of optic neuropathy related to vitamin B12 deficiency was
reported to originate from demyelination occurring in the papillomacular bundle, probably associated with synthesis of an incompatible fatty acid and cyanide accumulation. In addition, any damage in RGCs causes an intra-cellular superoxide explosion indicating later RGCs apoptosis. A recent research has shown that vitamin B12 functions as a superoxide cleaner with an effect similar to that of superoxide dismutase and could be preserved in cultured cells by neutralising superoxide. These results show that there could be an additional role beyond the co-factor functions of cobalamin and a function as an endogenous neuroprotectant for RGCs may be seen.

In the pediatric age group studies, it has been shown that there are neurologically negative effects on an infant during the lactation period, when the mother has vitamin B12 deficiency. Rasmussen et al. emphasised the importance of vitamin B12 in the development of the central nervous system, and stated that even a mild deficiency of vitamin B12 could be harmful in infancy and childhood. Furthermore, in the study of a pediatric age group by Özkasap et al., it was reported that vitamin B12 deficiency could have negative effects on the development of the central nervous system. Cortical atrophy, thinning of the corpus callosum and delayed myelination have been reported as neuro-radiological imaging findings in cases with vitamin B12 deficiency. In addition, it has recently been stated that bilateral abducens palsy, upward gaze palsy, total ophthalmoplegia, internuclear ophthalmoplegia and nystagmus could be related to vitamin B12 deficiency and this could be due to demyelination of the papillomacular bundle. Also, severe neurological disorders have been determined in adolescents following a strict vegetarian diet with vitamin B12 deficiency.

In addition, in a previous study conducted in Turkey using OCT, a relationship was shown between reduced superior RNFL thickness and vitamin B12 deficiency in a pediatric age group. In another OCT study by Turkyilmaz et al., the RNFL thickness in the temporal quadrant was found to be thinner in patients with vitamin B12 deficiency and was correlated to plasma B12 levels. In the current study, the superior RNFL and global RNFL thicknesses were measured as statistically significantly thinner than those of the control group and these measurements were significantly correlated with vitamin B12 level, which may associated with impaired myelination.

In some cases, neurological signs and symptoms may be early and the only findings of vitamin B12 deficiency. Neuropsychiatric symptoms may even be seen in hematologically normal cases. Although the mechanism of neurological symptoms in vitamin B12 deficiency is not clear, it has been reported that delayed myelination and the associated delayed synaptic connections could have a negative effect on brain development and function. Generally the spinal cord is affected first by neurological involvement of vitamin B12 deficiency. The term, subacute combined degeneration, has traditionally been defined for a spinal cord lesion of vitamin B12 deficiency and is useful in the differentiation from other spinal cord diseases including the posterior and lateral columns. The most common neurological symptoms associated with vitamin B12 deficiency are general weakness and paresthesia. Subsequently, findings can be seen that progress from hesitation when walking to ataxic paraplegia and mental symptoms such as irritability, apathy and sleepiness are also common. Sometimes, visual disturbance associated with optic neuropathy may be the earliest and only symptom. In the current cases, the most common symptoms were weakness and fatigue, dizziness and paresthesia. There were no visual symptoms in any of the current cases and no hematological abnormalities were determined.

Previous studies have shown that the prognosis of neurological involvement associated with vitamin B12 deficiency is related to the severity of the vitamin B12 deficiency and general deficiency duration but not to the symptom duration. A delay in diagnosis associated with this could be related to permanent neurological damage and the importance of early diagnosis has been emphasised. In the current study, the mean duration of symptoms of the patients was 6.2 months, and no significant correlation was observed between the duration of symptoms and RNFL thickness. Especially in the patients with vitamin B12 deficiency who presented with neurological symptoms, a significant decrease in RNFL was determined with OCT, and there were no findings such as demyelination, axonal degeneration and cortical atrophy on MRI. In the current study, the use of OCT, which has recently started to be widely used in the diagnosis of glaucoma and neurodegenerative diseases, has shown that it could be of guidance in the early stages of neurological involvement in vitamin B12 deficiency before the development of permanent sequelae.

The diagnosis of vitamin B12 deficiency is generally based on the measurement of serum or plasma vitamin B12 level, and a concentration <200pg/mL is defined as vitamin B12 deficiency. Biochemical measurement of vitamin B12 has 90%-95% sensitivity in showing clinical deficiency and is generally sufficient for diagnosis. However, the vitamin B12 level may be normal in approximately 50% of subclinical cases. The measurement of blood levels of methylmalonic acid and homocysteine, which are increased in the early stage of vitamin B12 deficiency is a more sensitive screening method for cobalamin deficiency. Rasmussen et al. recommended the measurement of
methylmalonic acid and total homocysteine levels to determine vitamin B12 deficiency in children. That the homocysteine and methylmalonic acid levels were not measured constitutes the most important limitation of this study. Another limitation of this study that we did not performed clinical symptoms and findings at the time of presentation of the control sample and the study sample size was relatively small.

When it is considered that a significant proportion of myelinisation of the brain occurs in the first 2 years of life and continues until puberty, vitamin B12 deficiency must be considered in childhood. Although treatment costs are low, there can be extremely severe complications such as irreversible neurological damage in delayed and untreated cases. Also, it is not known whether this damage responds to replacement therapy.

In conclusion, the current study can be considered of importance in respect of early diagnosis and treatment as it is one of only a few studies that have examined the effects of vitamin B12 deficiency on the eye and optic nerve in pediatric population. RNFL thickness should be evaluated in children suffer from weakness, dizziness and numbness in the hands and feet and detected low serum vitamin b 12 level. Low RNFL values in these patients may be a direct / indirect indicator of central nervous system involvement as we found correlation between vitamin B12 level and RNFL thickness in this current study. Further studies are needed to determine whether RNFL damage is reversible with replacement therapy.

Acknowledgments: no

Conflict of interest: Authors declared any conflict of interest.

Ethical Standards: All the procedures upon human participants within this study is compatible with the ethical standards of the national research committee as well as 1964 Helsinki Declaration and its later amendments.

Informed Consent: Each participant included in the study signed informed consent.

REFERENCES


