The Evaluation of Retinal Nerve Fiber Layer in Children with Vitamin B12 Deficiency

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ABSTRACT

Purpose: To evaluate the peripapiller 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 b 12 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**.**

INTRODUCTION

Vitamin B12, which functions as a significant co-enzyme in DNA synthesis, is necessary for the maturation of blood cells and to maintain the functions of the central nervous system. [1] It plays an important role in the transfer and methylation stages of methyl groups, which are required for the synthesis of neurotransmitters, choline, phospholipids, nucleotides and myelin. [2] The spinal cord, brain, optic nerves and peripheral nerve system can be affected by vitamin B12 deficiency.[3]

Although often ignored, critical vitamin and nutritional deficiencies can lead to visual problems.[4,5] Vitamin B12 deficiency in particular is known to cause bilateral optic neuropathy characterised by cecocentral scotoma and slowly developing optic atrophy, in addition to hematological, neurological and neuropsychiatric symptoms. [6,7] Optic neuropathy associated with vitamin B12 deficiency is rare but is an important clinical condition. In the pediatric age group, vitamin B12 deficiency is mostly caused by inadequate intake and malabsorption.[8] Optic neuropathy associated with vitamin B12 deficiency typically causes painless visual loss that progresses slowly over months or years. In cases that have been ongoing for a long time with delayed treatment, the axonal degeneration may be more severe.[9,10] Diagnosis may not always be easy, and permanent neurological sequelae may be seen associated with delayed treatment. [9] When diagnosis is made early it can be reversed with treatment.[11] Retinal nerve fiber layer (RNFL) thickness (µm) measurement by OCT may help the diagnosis and treatment follow up of these patients.

Optic coherence tomography (OCT) is a non-invasive imaging device designed for the high-resolution evaluation of the retina and RNFL thickness. It allows the quantitative measurement of the RNFL thickness and is useful in

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the identification of glaucoma patients. [12] 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 applied 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, intra-ocular 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

Table 1. *The demographic and clinical data of the patients and control group.*

	Patient group ± SD (n=26)	Control group ± SD (n=30)	P value
Age (year)	11.69 ± 3.77	10.90 ± 3.49	0.421
Gender (M/F)	15/11	15/15	0.573
Axial length (mm)	22.58 ± 0.90	22.67 ± 0.88	0.738
IOP (mmHg)	12.4 ± 1.61	12.3 ± 1.64	0.902
SE (Diopter)	-0.041	-0.23	0.094
Folic acid (ng/mL)	8.04 ± 2.39	8.09 ± 2.61	0.934
Vitamin B12 (pg /mL)	154.34 ± 17.84	267.90 ± 28.45	**<0.001***

M: male, F: female, IOP: intra-ocular pressure, SE; spherical equivalent, SD; standard deviation, * Statistically significant

examinations of the cases with a history of seizure and syncope, no abnormality was determined. The clinical symptoms and findings at the time of presentation of the cases with evident vitamin B12 deficiency are shown in Table 2.

The RNFL thickness measurements of the study group and the control group are shown in Table 3. In comparison with the control group values, the superior RNFL and global RNFL measurements of the study group were statistically significantly lower, indicating a thinner layer (p=0.01, p=0.007). The nasal and inferior RNFL measurements of the study group were lower than those of the control group, but no statistically significant difference was determined (p=0.069, p=0.097, respectively).

Table 2: *Clinical symptoms and findings at the time of presentation of the cases determined with vitamin B12 deficiency.*

Weakness and fatigue	96.2%
Dizziness	80.8%
Numbness in the hands and feet	30.8%
Seizure	19.2%
Syncope	15.4%
Abdominal pain	7.7%

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

Table 3. *Retinal nerve fiber layer thickness measurements of the study group and the control group.*

RNFL thickness (µm)	Patient group ± SD (n=26)	Control group ± SD (n=30)	P value
Temporal RNFL	79.34 ± 16.83	77.83 ± 8.72	0.682
Nasal RNFL	71.66 ± 15.67	78.50 ± 12.07	0.069
Superior RNFL	113.15 ± 18.96	129.56 ± 16.68	**0.001***
Inferior RNFL	124.23 ± 16.03	132.00 ± 18.06	0.097
Global RNFL	96.73 ± 11.18	104.43 ± 9.55	**0.007***

RNFL; retinal nerve fiber layer, SD; standard deviation, * Statistically significant.

reported to originate from demyelinisation occurring in the papillomacular bundle, probably associated with synthesis of an incompatible fatty acid and cyanide accumulation. [18] 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 [19] and could be preserved in cultured cells by neutralising superoxide. [20] 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. [21]

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. [22,23] Rasmussen et al. [24] 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.[25] 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 myelinisation have been reported as neuro-radiological imaging findings in cases with vitamin B12 deficiency. [26,27] 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 demyelinisation of the papillomacular bundle. [28] Also, severe neurological disorders have been determined in adolescents following a strict vegetarian diet with vitamin B12 deficiency.[29]

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.[25] 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.[10] 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 myelinisation.

In some cases, neurological signs and symptoms may be early and the only findings of vitamin B12 deficiency.[30] 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 myelinisation and the associated delayed synaptic connections could have a negative effect on brain development and function. [31] 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. [3] 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. [3] 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 [32] but not to the symptom duration. [11] A delay in diagnosis associated with this could be related to permanent neurological damage and the importance of early diagnosis has been emphasised. [11] 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. [33] Biochemical measurement of vitamin B12 has 90%-95% sensitivity in showing clinical deficency and is generally sufficient for diagnosis. [34] 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.[35] Rasmussen et al. recommended the measurement of

methylmalonic acid and total homocysteine levels to determine vitamin B12 deficiency in children. [24] 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 group 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.

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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

1. Soysal T. Megaloblastik Anemiler. İ.Ü. Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri. Anemiler Sempozyumu. İstanbul, 2001:33-47.
2. Shane B (2008) Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food Nutr Bull 29(2):5–16.
3. Miller A, Korem M, Almog R et al. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. J Neurol Sci. 2005 Jun 15;233(1-2):93-7.
4. Whatham A, Bartlett H, Eperjesi F et al. Vitamin and mineral deficiencies in the developed world and their effect on the eye and vision. Ophthalmic Physiol Opt. 2008 Jan;28(1):1-12.
5. Chew EY. Nutrition effects on ocular diseases in the aging eye. Invest Ophthalmol Vis Sci. 2013 Dec 13;54(14):42-7.
6. Larner AJ. Visual failure caused by vitamin B12 deficiency optic neuropathy. Int J Clin Pract. 2004 Oct;58(10):977-8.
7. Cohen H: Optic atrophy as the presenting sign in pernicious anemia, Lancet 1936, 228:1202-1203.
8. Babior BM, Bunn HF. Megaloblastic anemias. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine, 15th ed. New-York' McGraw-Hill; 2001. p. 674– 80.
9. Chavala SH, Kosmorsky GS, Lee MK et al. Optic neuropathy in vitamin B12 deficiency. Eur J Intern Med. 2005 Oct;16(6):447-8.
10. Türkyılmaz K, Öner V, Türkyılmaz AK et al. Evaluation of peripapillary retinal nerve fiber layer thickness in patients with vitamin B12 deficiency using spectral domain optical coherence tomography. Curr Eye Res. 2013 Jun;38(6):680-4.
11. Healton EB, Savage DG, Brust JC et al. Neurologic aspects of cobalamin deficiency. Medicine (Baltimore). 1991 Jul;70(4):229-45.
12. Budenz DL, Michael A, Chang RT et al. Sensitivity and specifcity of the Stratus OCT for perimetric glaucoma. Ophthalmology 2005;112: 3 – 9.
13. Pueyo V, Ara JR, Almarcegui C et al. Sub-clinical atrophy of the retinal nerve fbre layer in multiple sclerosis. Acta Ophthalmol 2010; 88: 748 – 752.
14. Berisha F, Feke GT, Trempe CL et al. Retinal abnormalities in early Alzheimer's disease. Invest Ophthalmol Vis Sci 2007; 48: 2285 – 2289.
15. Inzelberg R, Ramirez JA, Nisipeanu P et al. Retinal nerve fber layer thinning in Parkinson disease. Vision Res 2004;44: 2793 – 2797.
16. Svenson J. Neurologic disease and vitamin B12 defciency. Am J Emerg Med 2007;25:987.
17. Victor M, Ropper AH. Diseases of the nervous system due to nutritional deficiency. Adams and Victor's principles of neurology, 7th ed. New York' McGraw-Hill; 2001. p. 1218–22.
18. Hind VM. Degeneration in the peripheral visual pathway of vitamin B12-defcient monkeys. Trans Ophthalmol Soc U K 1970; 90: 839 – 846.
19. Birch CS, Brasch NE, McCaddon A et al. A novel role for vitamin B (12): Cobalamins are intracellular antioxidants in vitro. Free Radic Biol Med. 2009 Jul 15;47(2):184-8.
20. Suarez-Moreira E, Yun J, Birch CS et al. Vitamin B (12) and redox homeostasis: cob(II)alamin reacts with superoxide at rates approaching superoxide dismutase (SOD). J Am Chem Soc. 2009 Oct 28;131(42):15078-9.

21. Chan W, Almasieh M, Catrinescu MM et al. Cobalamin-Associated Superoxide Scavenging in Neuronal Cells Is a Potential Mechanism for Vitamin B (12)-Deprivation Optic Neuropathy. Am J Pathol. 2018 Jan;188(1):160-172.
22. Chester EM, Agamanolis DP, Harris JW et al. Optic atrophy in experimental vitamin B12 defciency in monkeys. Acta Neurol Scand 1980; 61:9 – 26.
23. Lövblad K, Ramelli G, Remonda L et al. Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatr Radiol. 1997 Feb;27(2):155-8.
24. Rasmussen SA, Fernhoff PM, Scanlon KS. Vitamin B12 deficiency in children and adolescents. J Pediatr. 2001 Jan;138(1):10-7.
25. Özkasap S, Türkyilmaz K, Dereci S et al. Assessment of peripapillary retinal nerve fiber layer thickness in children with vitamin B12 deficiency. Childs Nerv Syst. 2013 Dec;29(12):2281-6.
26. Biancheri R, Cerone R, Schiaffino MC et al. Cobalamin (Cbl) C/D deficiency: clinical, neurophysiological and neuroradiologic findings in 14 cases. Neuropediatrics 2001;32:14-22.
27. Rossi A, Cerone R, Biancheri R et al. Early-onset combined methylmalonic aciduria and homocystinuria: neuroradiologic findings. AJNR Am J Neuroradiol 2001;22:554-63.
28. Akdal G, Yener GG, Ada E et al. Eye movement disorders in vitamin B12 deficiency: two new cases and a review of the literature. Eur J Neurol 2007;14:1170-1172.
29. Ashkenazi S, Weitz R, Varsano I et al. Vitamin B12 deficiency due to a strictly vegetarian diet in adolescence. Clin Pediatr (Phila). 1987 Dec;26(12):662-3.
30. Lindenbaum J, Healton EB, Savage DG et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med. 1988 Jun 30;318(26):1720-8.
31. Irevall T, Axelsson I, Naumburg E. B12 deficiency is common in infants and is accompanied by serious neurological symptoms. Acta Paediatr. 2017 Jan;106(1):101-104.
32. Von Schenck U, Bender-Götze C, Koletzko B. Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. Arch Dis Child. 1997 Aug;77(2):137-9.
33. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr. 2009 Feb;89(2):693-6.
34. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr 2011;94:348–358.
35. Lindenbaum J, Savage DG, Stabler SP et al. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. Am J Hematol. 1990 Jun;34(2):99-107.