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Research Article

Evaluation of retinal nerve fiber layer and ganglion cell layer thicknesses with optical coherence tomography in patients with vitamin B12 deficiency

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ABSTRACT

Aim: We aimed to compare the retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) thickness of B12 vitamin deficiency patients with healthy controls using optical coherence tomography (OCT).

Methods: Forty-six patients (27 females / 19 males) diagnosed with B12 vitamin deficiency and 46 healthy controls (26 females / 20 males) with similar age and sex were included in the study. RNFL thickness of global, superotemporal, temporal, inferotemporal, superonasal, nasal and inferonasal sectors and GCL thickness and volume measurements of central, superior, inferior, temporal, and nasal sectors were performed using Spectralis-OCT device in all cases.

***Results*:** The mean age of the patient group was 42.17±15.34 years, while that of the control group was 44.21±12.34 years (p=0.528). Mean serum vitamin B12 levels were measured as 163,47±19,80 pg/ml in the patient group and 311,80±76,30 pg/ml in the control group (p <0,01). There was no statistically significant difference between the global RNFL thicknesses of the two groups (p> 0,05). However, statistically non-significant thinning was observed in the superotemporal and global RNFL thickness of the group with B12 vitamin deficiency (p values are 0,140 and 0,171, respectively). There was also no statistically significant difference between GCL thicknesses and volumes of the two groups (p> 0.05).

Conclusions: No significant reduction was observed in RNFL and GCL thicknesses of adult subjects with B12 vitamin deficiency compared with healthy controls.

Keywords: Ganglion cell layer; optical coherence tomography; retinal nerve fiber layer; vitamin B12 deficiency.

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Introduction

Vitamin B12 deficiency is a common disorder. Between 1999 and 2004, the prevalence of vitamin B12 deficiency was found to be 10.6% in the US population, when <200 pg/ml determined as the cutoff [1]. In Turkish population this rate was reported as 22.6% (For <200 pg/ml) [2]. Vitamin B12 deficiency is more common in elderly patients [3].

Vitamin B12 deficiency can be caused by a nutritional deficiency (vegetarian diet), cobalamin absorption disorders (intrinsic factor deficiency, malabsorption syndromes) cobalamin metabolite or transport disorders [3, 4]. Since vitamin B12 can be stored in the liver, kidney and other tissues, most patients may not present clinical signs and symptoms for up to 5-6 years after the development of deficiency [3].

Vitamin B12 is a cofactor for the synthesis of various neurotransmitters, choline, phospholipids, and nucleotides. Vitamin B12, which functions in the metabolism of homocysteine, energy, immunological system and nervous system, is especially important in the synthesis of DNA, erythrocyte and myelin sheath [5]. Hematologic, gastrointestinal, and neuropsychiatric disorders can occur in the deficiency of vitamin B12 [6]. Hematologic findings may also accompany neurological deficits in vitamin B12 deficiency. Neurological disorders caused by vitamin B12 deficiency include paresthesias, cognitive dysfunctions, dementia, brain atrophy, multiple sclerosis, optic neuritis and optic atrophy [7-9]. There are few studies in the literature investigating the visual effects of vitamin B12 deficiency [8-10]. The diagnosis of subclinical vitamin B12 deficiencies is usually delayed or overlooked [11]. In the case of delayed diagnosis and treatment, it may cause permanent neurological disorders [12]. It is recommended that vitamin B12 level should be examined in all patients with unexplained neuropsychiatric disorders [13].

Optical coherence tomography (OCT) is a non-invasive and non-contact method that allows high-resolution cross-sectional imaging of biological tissues. OCT allows detailed and precise evaluation of the retinal nerve fiber layer (RNFL) formed by the axons of the retina and retinal ganglion cells, and hereby, it is used for the diagnosis and follow-up of many neurodegenerative and neuro-ophthalmologic disorders and in particular to glaucoma [14, 15].

In the present study, we aimed to investigate the effect of vitamin B12 deficiency on the RNFL and ganglion cell layer (GCL) thickness, and also to evaluate whether there is a correlation between vitamin B12 level and RNFL thickness and GCL.

Methods

This prospective study included a total of 46 patients with vitamin B12 deficiency (<200 pg/mL) who were admitted to Abant Izzet Baysal University, Education and Research Hospital and a control group consisting of 46 healthy individuals between January 2015 and December 2016. The right eyes of both groups were examined. Detailed neurological examination and imaging were performed to all participants and no additional systemic or neurologic disease was detected. Written informed consent was obtained from all the participants included in the study. The study was approved by the local Ethics Committee (no: 2013-19) and conducted in accordance with the Declaration of Helsinki.

In all participants, detailed ophthalmologic examination was performed and visual acuity was measured with Snellen chart and also biomicroscopic and fundoscopic evaluations

of all were performed. The Canon TX-20P device (Canon Inc., Tokyo, Japan) was used to measure intraocular pressure (IOP) and central corneal thickness (CCT). Nidek ARK 510A auto refractometer (Nidek Co., Ltd., Aichi, Japan) was used to measure refraction values. Patients with corneal pathology, retinopathy, glaucoma, pseudoexfoliation syndrome, asthma, neurological disease other than B12 deficiency, peripheral vasospasm, previous history of eye surgery or eye trauma, more than 3 diopter myopia and hypermetropia were excluded.

OCT measurements were performed using a Spectralis OCT instrument (Spectralis®, Heidelberg Engineering, Heidelberg, Germany) loaded with a software version 5.3 by a single ophthalmologist (SA). Retinal thickness measurements were obtained from points at the center of the fovea, at 1500 µm from the fovea in its nasal macula, and at 1500 µm from the fovea in its temporal macula, with the "FAST" mode of the device. The measurements were performed using the default settings of the device. RNFL thickness analysis was performed in seven sectors using "Fast RNFL Thickness" mode; global (mean), superotemporal, temporal, inferotemporal, superonasal, nasal and inferonasal. Thickness measurements of GCL thickness were performed in the central, superior, inferior, temporal and nasal sectors.

Serum vitamin B12 levels were measured using Chemiluminescent Microparticle Immunoassay (Architect 2000; Abbot, Abbott Park, IL, USA). The limit value for vitamin B12 deficiency was determined as <200 pg/ml according to the manufacturer's instructions and the studies in the literature [1, 16].

Statistical Analysis

Statistical analysis of the data obtained from the study was performed using "SPSS for Windows 22.0" (SPSS Inc., Chicago, IL, USA). Variables were expressed as mean ± standard deviation and number (%). Independent sample t-test was used for comparison of groups. The Chi-square test was used to compare the distribution of gender between groups. Pearson's correlation test was used for correlation of parametric data, and Spearman's rho test was used for correlation of non-parametric data. A p value of less than 0.05 was considered statistically significant.

Results

Demographic and clinical parameters of the groups are shown in Table 1. The visual acuity of all cases was 10/10. There was no significant difference between the mean spherical equivalents of the two groups, IOP and Central corneal thickness (CCT) (p values of 0,488, 0,841 and 0,559, respectively).

There was no significant difference in the retinal thickness, choroidal thickness, RNFL thickness, and GCL volume values between the groups. The thickness of the superotemporal and global RNFL of the group with vitamin B12 deficiency was found to be lower than healthy participants. However, this thinning was not statistically significant (p-value of 0.140 and 0.171 respectively). There was no significant correlation between GCL thickness and vitamin B12 levels (Table 2). There was a significant correlation between serum vitamin B12 levels and the thickness of superotemporal RNFL (r = 0.38).

Discussion

The results of our study showed that the RSLT thickness, GCL volume, and thickness of the patients with vitamin B12 deficiency was not

Table 1. Comparison of demographic characteristics and clinical parameters of the groups.

Parameters	Patients with vitamin B12 deficiency (n=46)	Healthy controls (n=46)	P value
Age (years)	42,17±15,34	44,21±12,34	0,528
Gender (Female/male)	27/19	26/20	0,432
vitB12 (pg/ml)	163,47±19,80	311,80±76,30	<0,001
Seq	-0,31±1,53	0,05±0,94	0,488
IOP (mmHg)	15,41±3,87	15,64±2,67	0,841
CCT (µm)	555,47±27,14	549,14±17,76	0,559
RNFLT-g (µm)	100,32±7,24	103,09±10,44	0,171
RNFLT-st (µm)	136,98±17,68	143,03±17,90	0,140
RNFLT-t (µm)	79,30±11,10	76,65±11,06	0,299
RNFLT-it (µm)	151,34±14,64	155,68±15,33	0,208
RNFLT-sn (µm)	102,27±21,51	105,35±21,37	0,531
RNFLT-n (µm)	78,20±35,47	78,18±16,43	0,997
RNFLT-in (µm)	114,98±37,43	111,59±22,91	0,644
RT-f (µm)	218,04±21,81	221,50±26,07	0,521
RT-t (µm)	326,07±16,34	321,71±15,93	0,237
RT-n (µm)	347,22±15,59	348,53±14,61	0,704
GCLT-c (µm)	13,77±4,10	14,47±4,58	0,550
GCLT-s (µm)	54,36±4,75	53,68±4,08	0,590
GCLT-t (µm)	48,18±5,46	48,89±4,39	0,621
GCLT-i (µm)	53,53±4,32	54,21±3,98	0,564
GCLT-n (µm)	52,02±5,32	52,21±4,87	0,896
GCLT-mean (µm)	44,39±4,23	44,69±3,40	0,797

Values: Mean ±SD. Seq: Spheric equivalan, IOP: Introcular pressure, CCT: Central corneal thickness, RNFLT: Retinal fiber layer thickness (g: Global, st: Superotemporal, t: Temporal, it: Inferotemporal, sn: Superonasal, n: Nasal, in: Inferonasal), RT: Retinal thickness (f: fovea, t: Temporal, n: Nasal), GCLT: Ganglion cell layer thickness (c: central, s: Superior, t: Temporal, i: Inferior, n: Nasal).

Table 2. Correlation between serum vitamin B12 level and RNFL and GCL thickness.

Parameters	Serum vitamin B12 level	
	Correlation coefficient (r)	P value
RNFLT-g	0,20	0,30
RNFLT-st	0,38	0,04
RNFLT-t	0,01	0,61
RNFLT-it	0,05	0,81
RNFLT-sn	-0,02	0,90
RNFLT-n	-0,23	0,24
RNFLT-in	-0,21	0,28
GCLT-m	-0,23	0,28
GCLT-s	-0,22	0,29
GCLT-t	0,04	0,87
GCLT-i	-0,15	0,46
GCLT-n	-0,47	0,08
GCLT-ort	0,06	0,32

RNFLT: Retinal fiber layer thickness (g: Global, st: Superotemporal, t: Temporal, it: Inferotemporal, sn: Superonasal, n: Nasal, in: Inferonasal), RT: Retinal thickness (f: fovea, t: Temporal, n: Nasal), GCLT: Ganglion cell layer thickness (c: central, s: Superior, t: Temporal, i: Inferior, n: Nasal).

significantly different from the healthy controls.

Vitamin B12 is involved as a cofactor in the synthesis of myelin sheath, DNA, choline and phospholipids [5, 17]. In addition to hematological disorders, vitamin B12 deficiency has been associated with various neuropsychiatric and neuro-ophthalmologic diseases such as cognitive dysfunctions, dementia, ataxia, peripheral neuropathy, myelopathy, optic neuropathy, nystagmus, internuclear ophthalmoplegia [7, 8].

Nevertheless, there are few studies in the literature regarding the effects of vitamin B12 on optic nerve and visual pathway. Some of these studies have suggested that vitamin B12 deficiency is associated with deterioration in optic neuropathy and visual evoked potentials [8, 9, 18, 19]. However, vitamin B12 studies related to optic atrophy and degeneration are limited to experimental studies conducted in animals and studies in children population. There is not enough study in adult population [18-20].

The primary effect of vitamin B12 on the nervous system is that it contributes the production of myelin nerve sheath. It has been reported that a number of neurological and psychiatric manifestations related to the vitamin B12 deficiency are caused by deterioration of myelin [21]. Özkasap et al. [22] reported that the mean RNFL was thinner in children with vitamin B12 deficiency. This may have been due to fact that it damages the myelination in early childhood. Türkyılmaz et al. [23] reported that the mean RNFL and temporal RNFL were thinner in adults with deficiency of B12 vitamins, but in this study, the limit value for vitamin B12 deficiency determined as 189 pg/mL. Although reference intervals for serum levels of vitamin B12 vary widely in different clinics, a limit value of 200 pg/ml has been used in many studies [1, 16]. We also determined the limit value for vitamin B12 deficiency as 200 pg/mL. On the other hand, the study of Türkyılmaz et al. comprises relatively younger adults (mean age of the patient and control group is 33,1±6.5 years, in our study the mean age in the B12 group was 42,17±15,34 years, and 44,21±12,34 years for the control group). Additionally, the smoking status of the participants was not mentioned in

their study. Previous studies have shown that the RNFL thickness of smokers is significantly thinner than non-smokers at a significant level [24].

Vitamin B12 deficiency is often diagnosed by measuring serum or plasma vitamin B12 levels. Although the measurement of serum vitamin B12 levels for diagnosis of deficiency is a common, easy, and practical method, the metabolic active form of vitamin B12 cannot be measured with this test. Therefore, sometimes clinical findings and serum vitamin B12 levels may be incompatible [13]. The serum holotranscobalamin test provides a more accurate assessment by measuring the metabolically active form of vitamin B12 [11]. However, this test has newly come into use and is not yet widespread. Measurement of serum homocysteine levels and serum and/or urine methylmalonic acid levels are other adjunctive tests that can be used for vitamin B12 deficiency. There is a diagnostic value especially in patients who are thought clinically to be deficient in vitamin B12 but whose serum vitamin B12 level is normal. However, there may be false positive results in some acquired and genetic diseases [1, 25]. The most important limitations of our study are that only the serum levels of vitamin B12 were measured, duration of the deficiency was not mentioned and the number of cases was relatively low. The inability to show a statistically significant correlation may be secondary to a lack of power due to the small sample size. Further studies using other diagnostic tests on larger population are needed.

In conclusion, there was no significant difference between OCT and retinal nerve fiber layer and ganglion cell layer measurements in patients with vitamin B12 deficiency. There was a significant correlation between serum vitamin B12 levels and the thickness of superotemporal RNFL. Further studies investigating OCT findings in patients with vitamin B12 deficiency are needed.

Compliance with ethical statements

Conflicts of Interest: None.

Financial disclosure: None

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