Int Ophthalmol (2021) 41:3145–3151
https://doi.org/10.1007/s10792-021-01879-x

ORIGINAL PAPER

The effects of vitamin B12 deficiency on retina and optic disk vascular density

Erel Icel · Turgay Ucak

Received: 31 October 2020 / Accepted: 26 April 2021 / Published online: 28 May 2021

Abstract
Purpose In this study, we aimed to determine the alterations in the retina and peripapillary vascular density in patients with vitamin B12 deficiency.
Material and method The patients were divided into two groups regarding their vitamin B12 levels as the low vitamin B12 group (< 200 pg/ml) and the normal vitamin B12 group (≥ 200 pg/ml). The retinal nerve fiber layer thickness (RNFLT) and the central macular thickness (CMT) were measured through an SD-OCT analysis. The foveal avascular zone (FAZ), vessel density (VD) of the superficial capillary plexus (SCP), and deep capillary plexus (DCP) of the macula, and the VD of the radial peripapillary capillary plexus (RPCP) for the optic disk were determined by OCT-A.
Results Thirty-three patients were in the low vitamin B12 group and 54 were in the normal group. The mean RNFLT measurements were significantly lower in the low vitamin B12 group ($p = 0.001$). The RPCP values in all quadrants were significantly lower in the patients with vitamin B12 deficiency ($p = 0.001$); moreover, there was a significant increase in the FAZ value with a decrease in both superficial ($p = 0.001$) and deep ($p = 0.001$) VDs. There was a significant positive correlation between the serum vitamin B12 levels and the RPCP values and superficial and deep VDs, while the FAZ value was negatively correlated with the vitamin B12 levels.
Conclusion Vitamin B12 deficiency has diverse effects on ocular structures and retinal vasculature. Decreased VDs and increased FAZ may be associated with severe ocular alterations in the long term, which should be further investigated.

Keywords Vitamin B12 · Optical coherence tomography angiography · Vessel density · Foveal avascular zone · Retina

Introduction

Today, vitamin B12 (cobalamin) deficiency, associated with malabsorption secondary to gastric diseases, certain drugs, or inadequate dietary intake, is not a rare condition [1]. Vitamin B12 is an essential vitamin for neurological functions. Cyanocobalamin, which is a form of vitamin B12, is an important mediator of some enzymatic reactions in humans, including the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A and the conversion of homocysteine to

E. Icel
Department of Ophthalmology, Faculty of Medicine, Erzincan Binali Yıldırım University, Erzincan, Turkey
e-mail: dr_erel@hotmail.com

T. Ucak (✉)
Department of Ophthalmology, University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey
e-mail: turgayucak10@gmail.com

methionine. In vitamin B12 deficiency, methylmalonyl-CoA and homocysteine accumulate in the serum. Impaired methionine synthesis is associated with impaired myelin phospholipid synthesis causing neurological dysfunction. Moreover, deficiency in succinyl-CoA synthesis is associated with the generation of odd-chained fatty acids [2, 3].

Although vitamin B12 deficiency has been investigated in many diseases in the literature, the data regarding its ocular effects are limited. Optic neuropathy and dry eye disease have been reported as the main ocular manifestations of vitamin B12 deficiency [4, 5]. This deficiency has also been associated with many vascular diseases due to the accumulation of odd-chained fatty acids [6].

To the best of our knowledge, the effects of vitamin B12 deficiency on peripapillary vascular densities have not been studied before. In this study, we aimed to determine the alterations in the retina and peripapillary vascular density in patients with vitamin B12 deficiency.

Material and methods

This prospective, observational, cross-sectional study was conducted between November 2019 and March 2020 after receiving approval from the ethics committee of Erzincan University (33,216,249–604.01.02–E.48489 11/10/2019) and completed in agreement with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Study population

Patients who were admitted to the Ophthalmology department outpatient clinic of Erzincan University Hospital between November 2019 and March 2020 were included in the study, consecutively. The demographic data of all cases were recorded. Detailed ocular examinations were performed in all patients. Only the right eyes of all participants were included in the study. The inclusion criteria were as follows: age between 18 and 60 years, refractive error $\leq \pm 1$ D or axial length between 22 and 24 mm, visual acuity of 20/20, intraocular pressure ≤ 21 mmHg. Patients with any chronic systemic diseases affecting ocular structures, such as diabetes mellitus and rheumatologic diseases, those having a history of any ocular disease, those that had undergone any ocular surgical intervention, and those that presence of any ocular problem (such as cataract, ectatic corneal diseases, chorioretinal diseases, glaucoma, etc.), history of contact lens use were excluded from the study. Besides, participants were excluded from the study in the presence of conditions such as thyroid diseases, anemia, pregnancy, breastfeeding period, smoking, liver and kidney failure, chronic drug use, acute or chronic infection status, hypertension, cardiovascular diseases that have the potential to affect the measurements.

After eight hours of fasting, complete blood count (Sysmex XN-1000 Hematology System (Sysmex, Kobe, Japan)), serum folate, and vitamin B12 levels (Centaur XP (Siemens, Germany)) analyses were performed in all patients. The patients were divided into two groups according to their vitamin B12 levels as the low vitamin B12 group (< 200 pg/ml) and the normal vitamin B12 group (≥ 200 pg/ml).

A complete ophthalmologic examination was performed in all patients, including auto-refractometry, visual acuity, Goldmann applanation tonometry, and posterior segment examination by biomicroscopy. The intraocular pressure levels (Tonoref III, Nidek Co. Ltd., Aichi, Japan) were recorded. A slit-lamp biomicroscopic examination was performed. The retinal nerve fiber layer thickness (RNFLT) and the central macular thickness (CMT) were measured through an SD-OCT analysis (Nidek Co. Ltd., Aichi, Japan). The macular and peripapillary thicknesses were also measured using the SD-OCT device. Macular cube line scans were used to assess the ganglion cell layer thickness (GCLT).

The foveal avascular zone (FAZ), the vessel density (VD) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) of the macula, and the VD of the radial peripapillary capillary plexus (RPCP) for the optic disk were determined by optical coherence tomography angiography (OCT-A) (RS-3000 Advance, Nidek Co. Ltd., Gamagori, Japan).

Scan protocol

The Nidek RS-3000 Advance OCT system and updated AngioScan software were used to evaluate the SD-OCT and OCT-A images. The light source of this device has a wavelength of 880 nm and its optical resolution in tissue is 7 microns on the *Z*-axis, 20

microns on the *XY* axis, and a speed of 53,000 A-scans per second. The fovea was focused on using an OCT-A prototype internal fixation lamp and 3 × 3 mm macula cubes, each consisting of 256 B-scans. The scans comprised a 2.4 * 4 mm disk map centered on the optic disk. The tracing HD plus function of the Nidek RS-3000 Advance system reduces motion and blink artifacts. Using this device, all measurements were obtained automatically. The SCP was between the inner limiting membrane and the inner plexiform layer. For the superficial plexus, FAZ was automatically calculated (Fig. 1). The DCP was extending from the inner nuclear layer to the outer plexiform layer. VD was calculated as the percentage area occupied by the vessels with blood flow in the selected region [7]. The VD measurements were undertaken by the device based on the colored VD maps of the optical disk head and macula (ETDRS chart) (TSNIT chart) (Fig. 2a, b).

The SD-OCT and OCT-A measurements were performed by an experienced clinician. Both eyes were dilated with 1% tropicamide eye drop (Tropamid, Bilim Ilac Ltd, Istanbul, Turkey). In cases where the signal strength index quality was < 7/10, scanning was repeated. FAZ and VD in the SCP and DCP were only measured in the right eyes of all participants.

Statistical analyses

Statistical evaluation was performed using SPSS software (version 21.0; SPSS Inc, Chicago, IL). The results of the patients' age, laboratory data, and instrument measurements were expressed as mean ± SD. Normal data distribution was assessed using the one-sample Kolmogorov–Smirnov test. Comparisons between two groups were performed with the chi-square test or Student's *t* test. Correlation analyses were undertaken with Pearson's correlation analysis in all participants. The *G* * Power package program was used for the calculation of minimum sample size. The minimum sample size was calculated as 30, considering the effect size as 0.5 since there was no available data in the literature, the type I error rate = 0.05 and power = 95%. In all analyses, $P < 0.05$ was considered statistically significant.

Results

A total of 87 patients (55 male, 32 female) were included in the study. The serum vitamin B12 levels were lower than 200 pg/ml in 33 patients (20 male, 13 female) and normal in 54 (35 male, 19 female). The gender distribution of the groups was similar ($p = 0.69$) (Table 1). The demographic data and laboratory findings of the two groups are summarized in Table 1. There was no significant difference between the groups regarding age, gender, or serum hemoglobin and folate levels.

The comparisons of the ophthalmologic data between the groups are summarized in Table 2. The best-corrected visual acuity (BCVA) was 20/20 in all participants. There was no significant difference between the groups concerning intraocular pressure or CMT. The average, superior, nasal, and inferior RNFLT measurements were significantly lower in the low vitamin B12 group.

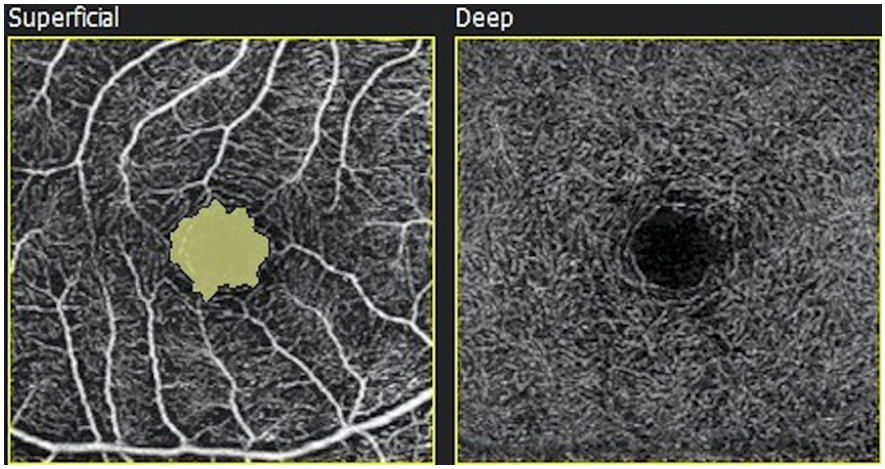

Fig. 1. Automatically calculated foveal avascular zone (FAZ) and superficial vascular density measurements of a participant, using Nidek's RS-3000 Advance and Navis Ex. Ver. 1.1.5 software

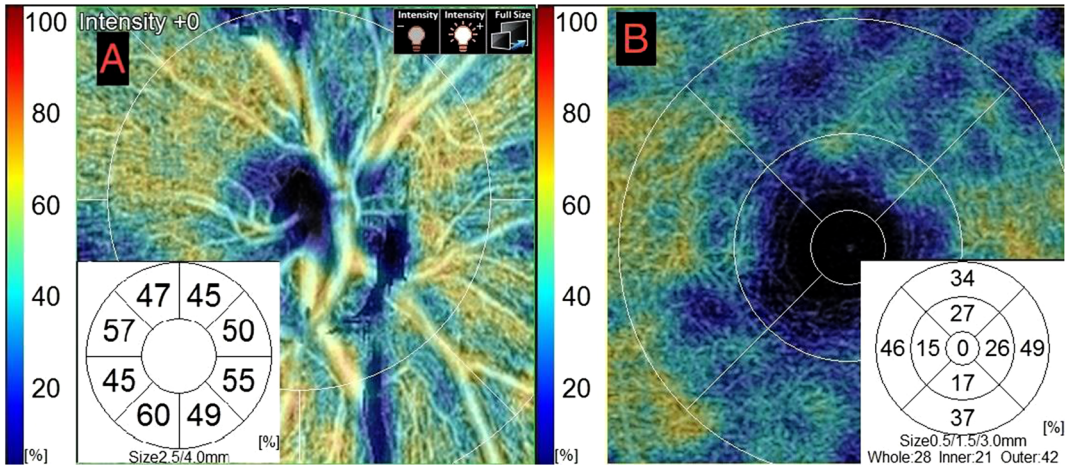

Fig. 2. **a** Color map of radial peripapillary capillary plexus (RPCP) and corresponding VD values, **b** Color map of vessel density (VD) at the level of deep capillary plexus and corresponding VD values

Table 1 Comparison of the demographical data and laboratory findings between the groups

	Vitamin B12 < 200 (*n*:33)	Vitamin B12 ≥ 200 (*n*:54)	*P**
Gender (M/F)	20/13	35/19	0.69
Age (years)	39.21 ± 11.02	42.00 ± 10.27	0.37
Hemoglobin (g/dL)	13.15 ± 0.91	13.60 ± 1.04	0.14
Folic acid (μmol/L)	9.44 ± 1.84	10.11 ± 1.98	0.12

M male, *F* female. *Significant *P* values shown in bold

Table 2 Comparison of the ophthalmologic data between the groups

	Vitamin B12 < 200 (*n*:33)	Vitamin B12 ≥ 200 (*n*:54)	*P**
IOP (mm-Hg)	13.48 ± 1.93	13.70 ± 2.54	0.67
RNFLT, average (μm)	98.59 ± 11.26	109.68 ± 9.28	**0.001**
Superior RNFLT (μm)	117.12 ± 16.38	137.22 ± 16.95	**0.001**
Nasal RNFLT (μm)	73.78 ± 19.66	85.33 ± 20.23	**0.011**
Inferior RNFLT (μm)	130.04 ± 15.53	139.78 ± 17.43	**0.034**
Temporal RNFLT (μm)	71.36 ± 11.29	76.61 ± 16.46	0.11
CMT (μm)	241.75 ± 63.19	226.35 ± 72.49	0.31
GCLT-superior (μm)	108.72 ± 20.54	111.96 ± 16.01	0.41
GCLT-inferior (μm)	96.33 ± 7.93	103.29 ± 6.16	**0.011**

IOP intraocular pressure, *RNFLT* retinal nerve fiber layer thickness, *CMT* central macular thickness, *GCLT* ganglion cell layer thickness. *Significant *P* values shown in bold

The comparisons of the OCT-A findings between the groups are summarized in Table 3. Scatter plots to show the relationship of individual vitamin B12 level to SCP, DCP and FAZ are presented in Fig. 3. The RPCP values in all quadrants were significantly lower in the patients with vitamin B12 deficiency; moreover, there was a significant increase in the FAZ value with a decrease in both superficial and deep VDs.

A correlation analysis was performed between the serum hemoglobin and vitamin 12 levels and OCT-A findings in all patients (Table 4). There was a significant correlation between all the OCT-A findings and the serum vitamin B12 levels.

Discussion

In this study, we analyzed the ocular alterations in vitamin B12 deficiency and found that although there was no significant change in intraocular pressure or

Table 3 Comparison of the OCT-A findings between the groups

	Vitamin B12 < 200 (*n*:33)	Vitamin B12 ≥ 200 (*n*:54)	*P**
RPCP VD, average (%)	48.03 ± 6.38	52.70 ± 4.68	**0.001**
Superior RPCP (%)	48.86 ± 6.96	54.44 ± 4.61	**0.001**
Nasal RPCP (%)	44.16 ± 6.71	49.53 ± 5.21	**0.001**
Inferior RPCP (%)	51.84 ± 7.57	56.87 ± 4.07	**0.001**
Temporal RPCP (%)	46.24 ± 8.64	51.73 ± 6.59	**0.001**
FAZ area (mm^2)	0.45 ± 0.11	0.31 ± 0.13	**0.001**
SCP VD, global (%)	37.00 ± 4.93	42.77 ± 4.35	**0.001**
DCP VD, global (%)	28.81 ± 8.04	36.57 ± 6.90	**0.001**

RPCP radial peripapillary capillary plexus, *FAZ* foveal avascular zone, *VD* vessel density, *SCP* superficial capillary plexus; *DCP* deep capillary plexus. *Significant *P* values shown in bold

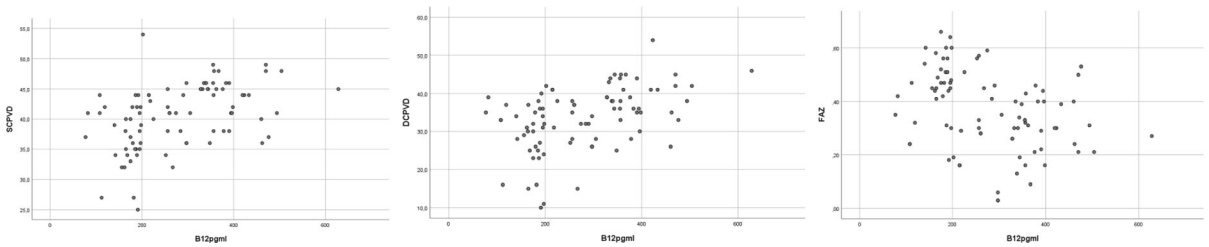

Fig. 3. **a** The association between vitamin B12 levels and superficial capillary plexus (SCP), **b** deep capillary plexus (DCP), **c** foveal avascular zone (FAZ)

Table 4 Correlation analysis between the serum hemoglobin and vitamin 12 levels and OCT-A findings in all patients

	Vitamin B12		Hemoglobin	
	R	*p**	*r*	*P**
RNFL	0.411	**0.001**	0.157	0.146
CMT	− 0.061	0.572	− 0.170	0.115
GCsup	0.082	0.449	0.192	0.078
GCinf	0.326	**0.002**	0.013	0.75
RPCP	0.370	**0.001**	0.078	0.471
FAZ	− 0.351	**0.001**	0.033	0.764
VD superficial	0.450	**0.001**	0.021	0.846
VD deep	0.452	**0.001**	0.026	0.812

RPCP radial peripapillary capillary plexus, *FAZ* foveal avascular zone, *VD* vessel density, *r* is the correlation coefficient and *p* is the significance level. *Significant *P* values shown in bold

CMT, the RNFLT and inferior GCLT values significantly decreased in the patients with low vitamin B12 levels. More importantly, for the first time in the literature, we determined a significant decrease in the RPCP values in all quadrants and a significant increase in the FAZ value with a significant decline in both superficial and deep VDs in the patients with vitamin B12 deficiency.

In previous literature, the data regarding the ocular alterations in vitamin B12 deficiency are limited. In this study, the intraocular pressure measurements did not significantly differ between the patients with low or normal serum vitamin B12 levels. Recently, similar to our results, in a population-based study of Leibovitzh et al. [8] conducted with 11,850 participants, no clinical correlation was observed between the homocysteine levels and intraocular pressure. In a meta-analysis, Li et al. [9] also reported that normal-tension glaucoma was not associated with elevated plasma homocysteine, serum folic acid, or vitamin B12 levels. In the current study, we excluded the patients with ocular diseases and those with glaucoma.

We determined a significant decrease in the average, superior, and nasal RNFLT values in the patients with vitamin B12 deficiency. In previous studies, supporting our findings, there was a decrease in the RNFLT in vitamin B12 deficiency [10, 11]. Similar to

our results, Coskun et al. [12] also reported a significant decrease in the RNFLT of patients with iron deficiency anemia and vitamin B12 deficiency. Moreover, Srivastav et al. [13] reported a negative correlation between the homocysteine levels and RNFLT in patients with diabetic retinopathy. In vitamin B12 deficiency, anemia or decreased oxygen transport may be suggested to be responsible for the decrease in RNFLT; however, in our study, there was no significant difference between the groups regarding the hemoglobin levels. For this reason, we consider that the endothelial alterations in vitamin B12 deficiency develop before the manifestation of anemia. This thinning may be associated with the impairment of axonal transport due to a decline in oxidative phosphorylation and myelination.

We did not determine any significant difference in CMT according to the vitamin B12 levels. Dong et al. reported an increase in the central subfield macular thickness and average macular thickness with an increase in the plasma homocysteine levels in diabetic patients [14]. However, the data regarding the CMT alterations in nutritional anemia are limited, requiring further investigations.

We observed a significant decrease in the inferior GCLT, without any significant alterations in the superior GCLT in the patients with vitamin B12 deficiency. To the best of our knowledge, there is only limited research in the literature investigating the alterations in GCLT in vitamin B12 deficiency. Elevated homocysteine levels have been associated with apoptosis in retinal ganglion cells [15, 16].

Decreased vitamin B12 levels may be associated with augmented oxidative stress, which may be the reason for retinal ganglion cell loss and decreased GCLT in vitamin B12 deficiency [17]. For the first time in the literature, we evaluated the ocular vasculature in patients with vitamin B12 deficiency and determined a significant decrease in the RPCP values in all quadrants. This decrease in the capillary plexus may be the reason for the alterations we found in the retinal layers and described above. Moreover, we determined a significant increase in the FAZ values in the patients with vitamin B12 deficiency with a significant decrease in the superficial and deep VDs compared to those with normal serum vitamin B12 levels. Systemic hypoxia is known to affect retinal perfusion and retinal ganglion cells. In this study, there was no significant difference between the hemoglobin levels of the patients with normal and low vitamin B12 values. However, in the correlation analysis, we determined a significant correlation between RPCP, VD, and serum vitamin B12 levels. We determined that as the serum vitamin B12 levels decreased, the RPCP and VD values also decreased. In chronic hypoxic conditions, deficiencies in nutrients or oxygen and more importantly increased production of reactive oxygen species may cause severe problems in ocular tissues, which are highly susceptible to oxidative stress [18, 19]. Although the exact mechanism of these alterations in ocular vasculature is not known, we can suggest that vitamin B12 deficiency may be associated with increased oxidative stress altering ocular vasculature. However, further investigations are warranted to elucidate the pathophysiological mechanisms of these alterations.

There are some limitations to this study that should be mentioned. First, we did not investigate the duration of vitamin B12 deficiency or disease and we also do not know if these alterations occurred in the short or long term. Thus, further studies, evaluating the effects of vitamin B12 deficiency at different time points, are warranted. Second, we did not analyze the molecular aspect of these alterations, which can be the topic of another study. And lastly, we did not perform the visual field analysis, to see whether the changes in structures are related to the visual function, which may be the subject of another study.

In conclusion, vitamin B12 deficiency has diverse effects on ocular structures and retinal vasculature. Decreased VDs and increased FAZ may be associated with severe ocular alternations in the long term, which should be further investigated. Since vitamin B12 deficiency is not a rare disease but a treatable disease, the possibility of these alterations in ocular structures should be considered. Vitamin B12 levels should be examined in suspected patients to prevent further alterations in ocular structures. Considering that such cross-sectional studies will be incomplete in determining the effect of vitamin B12 level deficiency on the microvascular structure measured by OCTA, more precise results can be achieved by examining OCTA findings after vitamin B12 treatment with larger patient groups with follow-up studies in the future.

Authors' contributions IE planned the study, UT and IE contributed to the literature search and data extraction together, UT drafted and submitted the manuscript.

Funding None.

Data availability All data and material are available if required.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

Consent to publish Patients signed informed consent regarding publishing their data and photographs.

Ethical approval This cross-sectional study was conducted between November 2019 and March 2020 after receiving approval from the ethics committee of Erzincan University (33216249–604.01.02–E.48489 11/10/2019). All procedures performed in studies involving human participants were following the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All patients provided written informed consent.

References

1. O'Leary F, Samman S (2010) Vitamin B12 in health and disease. Nutrients 2(3):299–316
2. Langan RC, Goodbred AJ (2017) Vitamin B12 deficiency: recognition and management. Am Fam Physician 96(6):384–389
3. Sloan JL, Achilly NP, Arnold ML, Catlett JL, Blake T, Bishop K, Jones M, Harper U, English MA, Anderson S et al (2020) The vitamin B12 processing enzyme, mmachc, is essential for zebrafish survival, growth and retinal morphology. Hum Mol Genet 29(13):2109–2123
4. Sethi HS, Naik M, Gandhi A (2020) Megaloblastic anemia and bilateral disc edema: an enigma… have we figured it out yet? Taiwan J Ophthalmol 10(1):71–75
5. Ozen S, Ozer MA, Akdemir MO (2017) Vitamin B12 deficiency evaluation and treatment in severe dry eye disease with neuropathic ocular pain. Graefes Arch Clin Exp Ophthalmol 255(6):1173–1177
6. O'Lear F, Samman S (2010) Vitamin B12 in health and disease. Nutrients 2(3):299–316
7. Yilmaz H, Karakurt Y, Icel E, Ugurlu A, Ucak T, Tasli NG, Elpeze SE (2019) Normative data assessment of vessel density and foveal avascular zone metrics using angioscan software. Curr Eye Res 44(12):1345–1352
8. Leibovitzh H, Cohen E, Levi A, Kramer M, Shochat T, Goldberg E, Krause I (2016) Relationship between homocysteine and intraocular pressure in men and women: a population-based study. Medicine (Baltim) 95(38):e4858
9. Li J, Xu F, Zeng R, Gong H, Lan Y (2016) Plasma homocysteine, serum folic acid, serum vitamin B12, serum vitamin B6, MTHFR, and risk of normal-tension glaucoma. J Glaucoma 25(2):e94–e98
10. Özkasap S, Türkyilmaz K, Dereci S, Öner V, Calapoğlu T, Cure MC, Durmus M (2013) Assessment of peripapillary retinal nerve fiber layer thickness in children with vitamin B12 deficiency. Childs Nerv Syst 29(12):2281–2286
11. Türkyılmaz K, Öner V, Türkyılmaz AK, Kırbaş A, Kırbaş S, Şekeryapan B (2013) Evaluation of peripapillary retinal nerve fiber layer thickness in patients with vitamin B12 deficiency using spectral domain optical coherence tomography. Curr Eye Res 38(6):680–684
12. Coskun M, Sevencan NO (2018) The evaluation of ophthalmic findings in women patients with iron and vitamin b12 deficiency anemia. Transl Vis Sci Technol 7(4):16
13. Srivastav K, Saxena S, Mahdi AA, Shukla RK, Meyer CH, Akduman L, Khanna VK (2016) Increased serum level of homocysteine correlates with retinal nerve fiber layer thinning in diabetic retinopathy. Mol Vis 2(22):1352–1360
14. Dong N, Shi H, Tang X (2018) Plasma homocysteine levels are associated with macular thickness in type 2 diabetes without diabetic macular edema. Int Ophthalmol 38(2):737–746
15. Kern TS, Du Y, Miller CM, Hatala DA, Levin LA (2010) Overexpression of Bcl-2 in vascular endothelium inhibits the microvascular lesions of diabetic retinopathy. Am J Pathol 176(5):2550–2558
16. Ganapathy PS, Perry RL, Tawfik A, Smith RM, Perry E, Roon P, Bozard BR, Ha Y, Smith SB (2011) Homocysteine-mediated modulation of mitochondrial dynamics in retinal ganglion cells. Invest Ophthalmol Vis Sci 52(8):5551–5558
17. Van de Lagemaat EE, de Groot LCPGM, van den Heuvel EGHM (2019) Vitamin B12 in relation to oxidative stress: a systematic review. Nutrients 11(2):E482
18. Chrysostomou V, Rezania F, Trounce IA, Crowston JG (2013) Oxidative stress and mitochondrial dysfunction in glaucoma. Curr Opin Pharmacol 13(1):12–15
19. Chidlow G, Wood JPM, Casson RJ (2017) Investigations into hypoxia and oxidative stress at the optic nerve head in a rat model of glaucoma. Front Neurosci 24(11):478