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Corresponding Author:
Dong Cheol Lee, MD, PhD
Department of Ophthalmology, Keimyung University School of Medicine, Dongsan Medical Center, 1035, Dalgubeol-daero, Dalseo-gu, Daegu 42601, Korea
Tel: +82-53-258-4545
Fax: +82-53-258-4558
E-mail: tking33@naver.com

Retinal and Choroidal Vasculature Before and After Patch Occlusion Treatment Using Optical Coherence Tomography Angiography in Patients with Amblyopia

Jae-Gon Kim, Se Youp Lee, Dong Cheol Lee

Department of Ophthalmology, Keimyung University School of Medicine, Daegu, Korea

This study aimed to investigate structural differences in the retinal and choroidal blood vessels before and after patch occlusion treatment in patients with amblyopia using optical coherence tomography angiography (OCTA) and to determine whether these differences are related to clinical improvement in patients. A total of 26 eyes of 17 patients with monocular or binocular amblyopia who underwent patch occlusion treatment for at least 1 month were retrospectively enrolled. The width of the foveal avascular zone, retinal blood vessel density, choroidal thickness, and choroidal vascularity index (CVI) measured by OCTA were compared before and after the treatment; the correlation with change in best-corrected visual acuity (BCVA) was analyzed for the data showing statistically significant differences. The mean BCVA of amblyopic eyes before and after patch occlusion treatment was 0.41 ± 0.23 and 0.25 ± 0.16 in logarithm of the minimum angle of resolution units, respectively. A decrease of about 2% in CVI was observed after the treatment (p = 0.011). The correlation between the changes in CVI and the changes in BCVA were insignificant (Rs = 0.086, p = 0.718). The results indicated that a decrease in CVI was observed after the patch occlusion treatment, but the relationship between CVI and BCVA could not be established. Patch occlusion treatment in amblyopia appears to affect the changes in the choroidal vessels and stroma.

Keywords: Amblyopia, Choriocapillaris, Optical coherence tomography, Retinal vasculatures

Introduction

Amblyopia is defined as the reduction of best-corrected visual acuity (BCVA) in one or both eyes that cannot be attributed exclusively to a structural abnormality [1]. The treatment for amblyopia includes correction of refractive error, and patch occlusion or mydriasis of the dominant eye. The patching aims to restore normal or near-normal visual function by stimulating the amblyopic eye [1,2]. Through several animal experiments, it is presumed that patch occlusion treatment restores vision via development of more extensive synaptic input to the visual cortex, by stimulating the amblyopic eye during the period of visual development [3,4]. The effect of patch occlusion treatment in patients with amblyopia can be confirmed through measurement of BCVA [2]. However, in infants or children with disorders such as mental retardation or cerebral palsy, BCVA may not be accurately measured because of poor cooperation and communication.

Meanwhile, Optical coherence tomography angiography (OCTA) is a non-invasive technique to obtain images of the retinal and choroidal vasculature. Through OCTA, the information on vessel density (VD) and foveal avascular zone (FAZ) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) of the retina as well as the choroidal thickness (CT) can be obtained [5]. OCTA can be performed even in patients who are uncooperative or unable to concentrate; the images can be obtained, without any communication, if the patient can stare at a small target light for a relatively short period of about 30s. Therefore, the difference in retinal and choroidal blood vessels, before and after patch occlusion treatment, can be confirmed through OCTA even in patients for whom BCVA is difficult to measure.

The purpose of this study was to compare the retinal and choroidal blood vessels in amblyopic eyes, before and after patch occlusion treatment using OCTA, and to determine whether these differences were correlated with changes in BCVA.

Materials and methods

This study was approved by the Keimyung University Dongsan Hospital Institutional Review Board on August 04, 2020 (Approval number: 2020-07-098), and it adhered to the tenets of the Declaration of Helsinki and followed all guidelines for experimental investigation in human subjects.

In this retrospective study, patients with amblyopia who initially visited Keimyung University Dongsan Hospital (Daegu, Korea) between January 1, 2017, and July 15, 2020, and underwent monocular or binocular alternate patch occlusion treatment for at least 1 month, were enrolled. Uncooperative patients, identified through an interview of guardians or patients, and in whom BCVA could not be accurately measured, were excluded from this study. According to the cause of amblyopia, the patients were classified in three groups: strabismic amblyopia, refractive amblyopia, and combined (strabismic + refractive) amblyopia [2]. Patients with organic amblyopia or ophthalmic diseases other than amblyopia or systemic diseases were excluded from this study. Patients with visual deprivation amblyopia caused by congenital cataract, corneal opacity, and eyelid ptosis were also excluded [2]. Finally, a total of 17 patients (26 eyes with amblyopia) were enrolled as subjects for this study.

Clinical data collection

On the day of patch occlusion treatment initiation and the first follow-up, cycloplegic refraction, measured as spherical equivalent, with 1% cyclopentolate hydrochloride eye drop was performed. The Stereo Fly stereoscopic test was conducted to measure stereoscopic acuity. Patching was performed in the eye with better VA in cases of monocular amblyopia and alternated between eyes at daily intervals in cases of binocular amblyopia. The daily treatment time for each patient and amblyopic eye were recorded. In the case of binocular amblyopia, alternate patch occlusion treatment was performed; thus, the daily treatment time for each amblyopic eye was calculated as half of the alternate patch occlusion treatment time. For patients who had refractive or combined amblyopia, optical correction was prescribed before or at the time of patch occlusion treatment onset, which followed the Preferred Practice Pattern guidelines published by the American Academy of Ophthalmology in 2018[6]. The prescription of glasses was based on the results of the cycloplegic refraction; no other treatment was added until the first follow-up.

OCTA Data Collection

OCTA was performed for the amblyopic eyes of all included patients using a spectral-domain device DRI OCT Triton Plus (Topcon Co., Tokyo, Japan) on the day of patch occlusion and first follow-up after the treatment. The parameters of the flow areas in the fovea-centered 6 × 6 mm scan size wer e measured by built-in optical software (IMAGEnet 6 version 1.25.16650, Topcon Co., Tokyo, Japan). The period from the start of patch occlusion therapy to the first follow-up was different for each patient and recorded. The collected data included age, sex, amblyopic laterality (monocular or binocular), type of amblyopia, daily treatment time, follow-up period, BCVA as logarithm of the minimum angle of resolution units (LogMAR), spherical equivalent, stereoscopic acuity, the area of superficial FAZ and deep FAZ, VD of SCP (SCPD) that includes vasculature from the nerve fiber layer and ganglion cell layer, and VD of DCP (DCPD) that includes both the intermediate and deep inner retinal vasculature, CT, and choroid vascularity index (CVI) on the day of treatment initiation and first follow-up. The width of SFAZ, DFAZ, and CT were measured manually by the investigator through the IMAGEnet program, and CVI was measured manually using the ImageJ program (version 1.52a, National Institutes of Health, Bethesda, Maryland, USA).

FAZ was manually measured in SCP and DCP by drawing the boundary of the dark avascular area and recording SFAZ and DFAZ, respectively [7] (Fig. 1). VD was measured separately in SCP (SCPD) and DCP (DCPD) and within 1-mm, and 1-3mm diameter area based on macula, thus a total of four items was recorded: 1-mm fovea SCPD, 3-mm parafovea SCPD, 1-mm fovea DCPD, and 3-mm parafovea DCPD [8] (Fig. 2). CT was measured manually from the beginning of the choriocapillary layer, just below the retinal pigment epi-

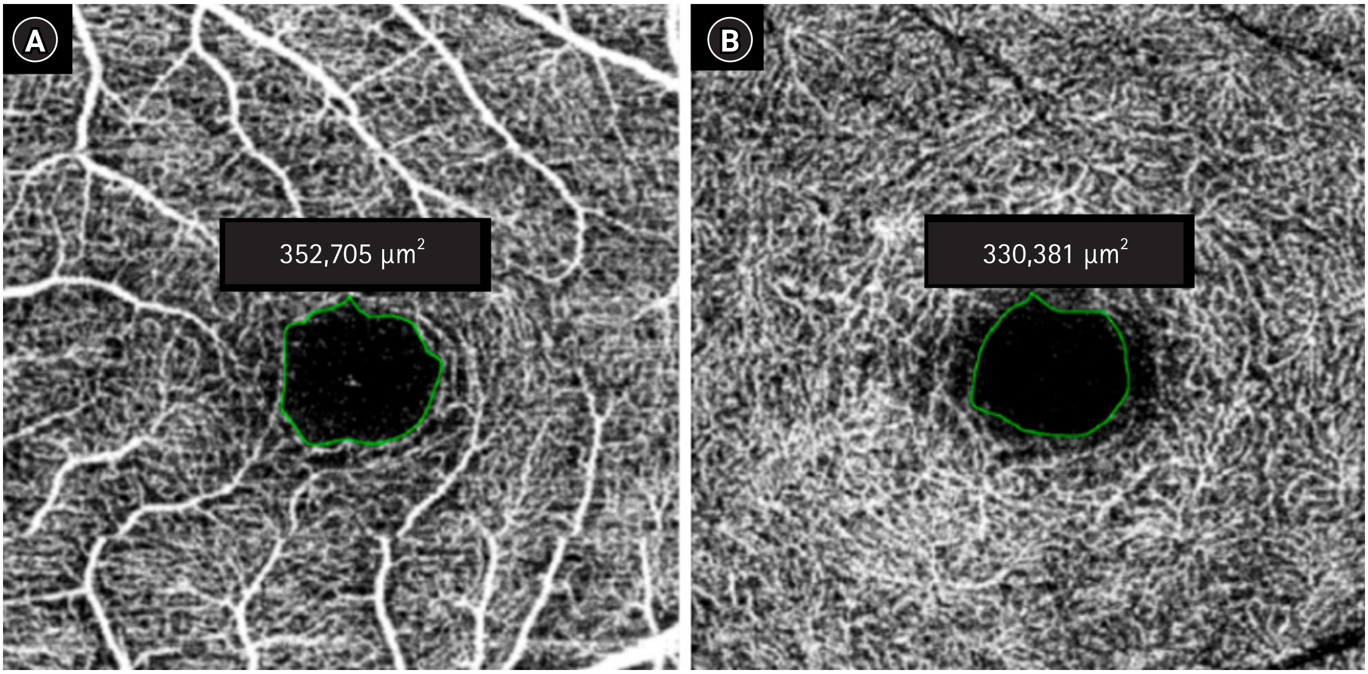

Fig. 1. Width of superficial foveal avascular zone (SFAZ) and deep foveal avascular zone (DFAZ) measured using the IMAGEnet program in a 7-year-old girl. (A) Width of SFAZ of the amblyopic eye (left eye: 352,705 μm^2). (B) Width of DFAZ of the amblyopic eye (left eye: 330,381 μm^2). The images were taken on the day of initiating patch occlusion treatment.

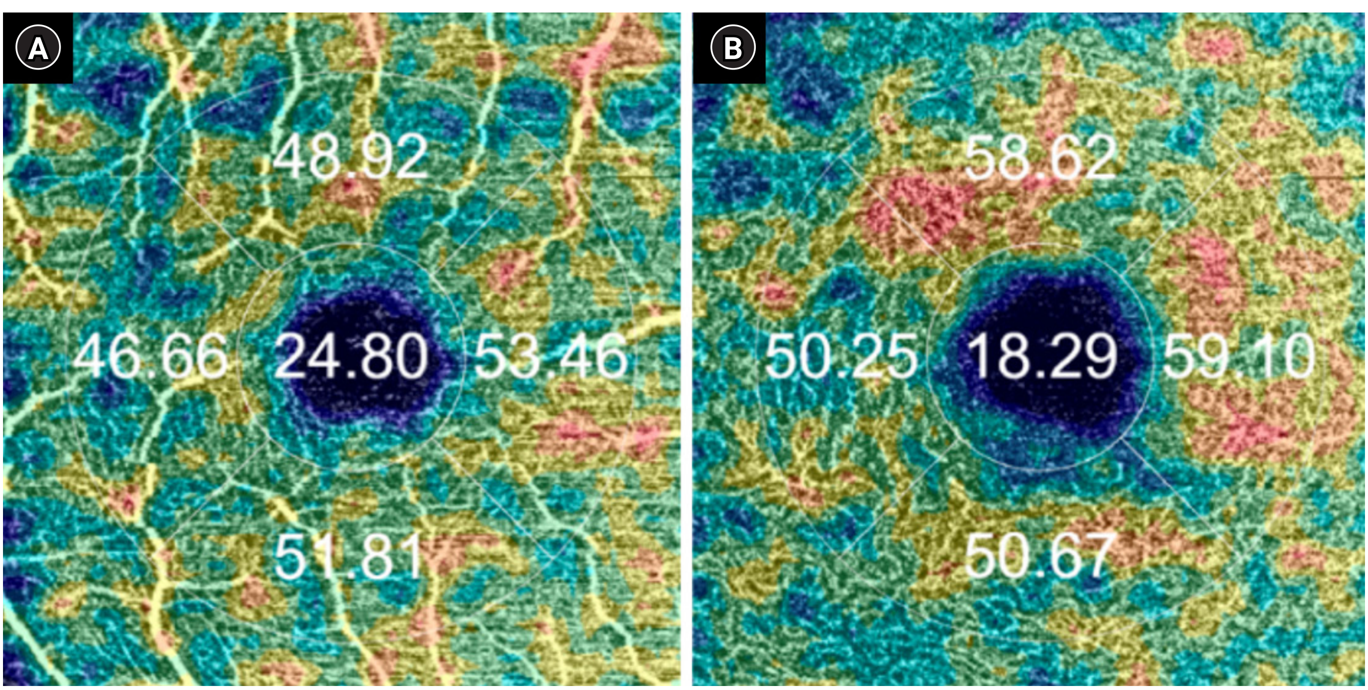

Fig. 2. One-mm fovea superficial capillary plexus density (SCPD), 3-mm parafovea SCPD, 1-mm fovea deep capillary plexus density (DCPD), and 3-mm parafovea DCPD measured using the IMAGEnet program in a 7-year-old girl. (A) SCPD of the amblyopic eye (right eye). (B) DCPD of the amblyopic eye (right eye). The center number represents the percentage of SCPD and DCPD inside a 1-mm-diameter circle from the fovea, and outer four numbers represent the percentage of SCPDs and DCPDs inside the circle 1 mm to 3 mm diameter from the fovea divided into four divisions: top, bottom, temporal, and nasal. One-mm fovea SCPD and DCPD is represented by the center number, and the 3-mm parafovea SCPD and DCPD is represented by the mean of the 4 numbers except 1-mm fovea SCPD. The images were taken on the day of initiating patch occlusion treatment. (1-mm fovea SCPD: 24.80%, 3-mm parafovea SCPD: 50.21%, 1-mm fovea DCPD: 18.29%, 3-mm parafovea DCPD: 54.66%).

thelial-Bruch's membrane complex, to the choroid-sclera boundary [9,10] (Fig. 3). CVI is the percentage of area occupied by blood vessels within a certain choroidal region [11]. Centered at the macula, a choroidal region of 750 µm in the temporal and nasal directions, with a total range of 1500 µm, was used as a reference of CVI [11] (Fig. 4). All data were measured by one researcher (J-G Kim).

Statistical Analysis

For statistical methods, all comparative variables before and after patch occlusion treatment were compared using the paired t-test if normality was satisfied in the Shapiro-Wilk test, and the Wilcoxon signed-rank test if normality was unsatisfied. The analyses were performed using IBM SPSS Statistics 25.0.0 (IBM Co., Armonk, NY, USA). Among them, only the data showing statistically significant changes were checked for the relationship with improvement in BCVA, using either Spearman's or Pearson's correlation analysis based on the normality. A p-value of < 0.05 was considered statistically significant.

Results

A total of 17 subjects with 26 eyes were enrolled. Nine had binocular amblyopia and eight had monocular amblyopia. Daily patching was implemented for an average of 1.6 ± 0.8 h for each amblyopic eye. The average follow-up period was 5.3 ± 4.7 months. Two patients had strabismic amblyopia and two had combined amblyopia; the rest had refractive amblyopia. The baseline characteristics of patients are shown in Table 1.

BCVA and stereoscopic acuity were significantly improved after the patch occlusion treatment ($p < 0.001$, $p = 0.019$, respectively). The spherical equivalent decreased due to the axial elongation during the normal development process of children [12], although the change was not statistically significant ($p = 0.055$) (Table 2).

Table 1. Baseline characteristics of the patients

Total number of subjects		17
Age (years)		6.4 ± 1.7 (4–9)
Sex (male:female)		9:8
Amblyopic type (monocular:binocular)		8:9
Mean daily treatment time (hours/day)		1.6 ± 0.8 (1-4)
Mean follow-up period (months)		5.3 ± 4.7 (1-16)
Type of amblyopia	Strabismic amblyopia	2
	Refractive amblyopia	13
	Combined amblyopia	2

Values are presented as mean ± standard deviation (range) or number.

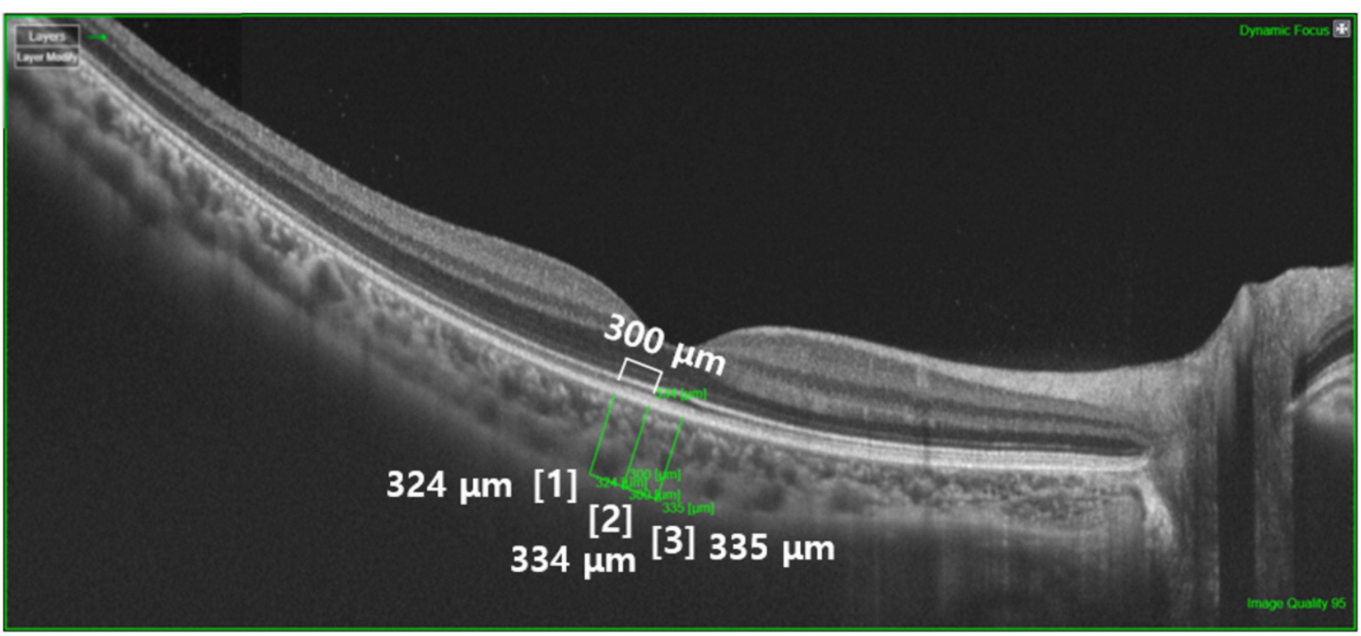

Fig. 3. Choroidal thickness (CT) measured using the IMAGEnet program in a 5-year-old boy. CT of the amblyopic eye (right eye). CT was measured from the beginning of the choriocapillary layer just below the retinal pigment epithelial-Bruch's membrane complex to the choroid-sclera boundary from the surface of the retina with respect to the macula. The CT was remeasured at a point 300 µm nasal and temporal from the macula, and the value was set as the mean of data measured at all three positions. The image was taken on the day of initiating patch occlusion treatment (temporal [1]: 324.00 µm, foveal [2]: 334.00 µm, nasal [3]: 335.00 µm, mean: 331.00 µm).

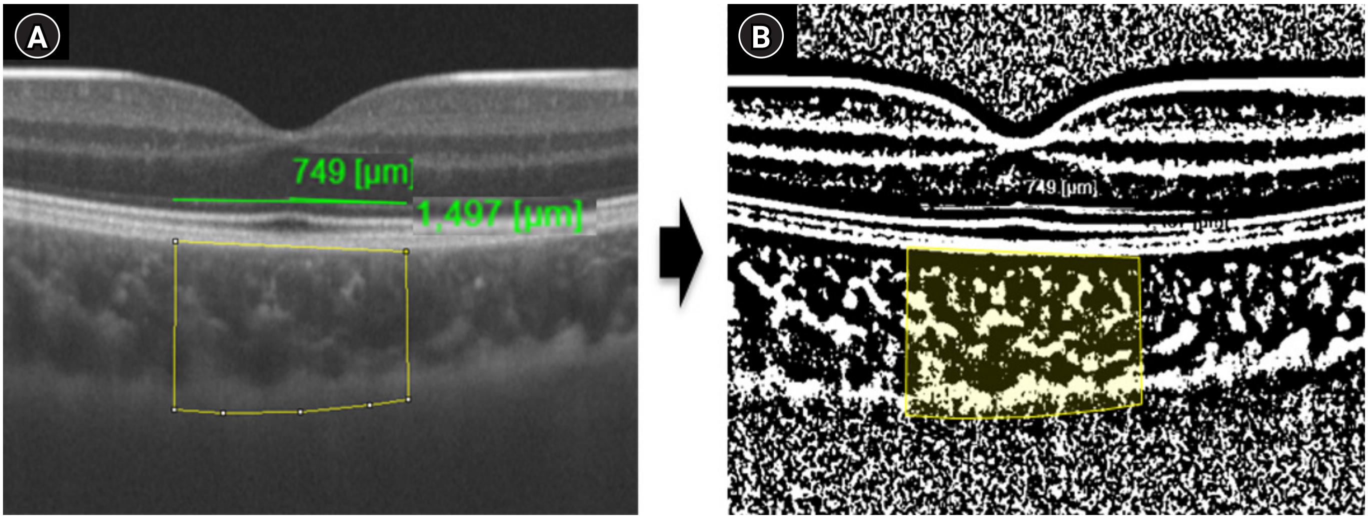

Fig. 4. Choroidal vascularity index (CVI) measured using the ImageJ program in a 9-year-old boy. (A) Transverse vertical section optical coherence tomography angiography image including the macula of amblyopic eye (right eye). A total choroidal area of 1,500 μm was taken as the basis of 750 μm to the nasal and temporal sides of the macula (the area inside the yellow border). (B) The image is binarized by Niblack's method of ImageJ program. The ratio of the black pixels (vascular area) to the black pixels + white pixels (total area) inside the yellow area was calculated (number of black pixels: 19,842, number of total pixels: 29,306, CVI: 67.71%).

Table 2. Comparison of best-corrected visual acuity (BCVA), stereoscopic acuity, and spherical equivalent before and after patch occlusion treatment

	Before occlusion treatment	After occlusion treatment	*p*-value
BCVA (by LogMAR, N = 26)	0.41 ± 0.23	0.25 ± 0.16	**< 0.001***
Stereoscopic acuity (seconds of arc, N = 12)	401.7 ± 827.9	82.5 ± 64.1	**0.019***
Spherical equivalent (D, N = 26)	-0.04 ± 2.55	-0.47 ± 2.98	0.055

Values are presented as mean ± standard deviation or number. Bold text indicates a statistically significant difference. LogMAR, logarithm of the minimum angle of resolution units; D, diopter. *p*-values were calculated using Wilcoxon Signed-Rank test. *$p < 0.05$

The width of the FAZ was measured in all but for 7 eyes. SCPD and DCPD were measured in 21 eyes and CT and CVI in 20 eyes, excluding those with examination errors. On comparison before and after patch occlusion treatment, CVI of the amblyopic eye was found to be decreased significantly after the treatment (p = 0.011 by paired t-test). The mean CVI on the day of treatment initiation and first follow-up was 64.49% ± 2.42% and 62.31% ± 2.50%, respectively. When comparing the other variables, before and after patch occlusion treatment, there was no significantly improved variable (Table 3).

Correlation was analyzed by setting the degree of change in CVI (CVI after patching–CVI before patching) and BCVA (BCVA after patching–BCVA before patching, by LogMAR) as variables. They seemed to show a weak positive correlation that was not statistically significant (Rs = 0.086, p = 0.718, Fig. 5).

Discussion

Several studies have investigated the changes in the blood vasculature in the retina and choroid associated with the treatment of amblyopia. Particularly, the results of studies on the change in CT according to treatment are controversial. Öner and Bulut [13] and Araki et al [14] reported that there was no significant difference in CT before and after refractive correction and patch occlusion treatment for amblyopia in amblyopic eyes, which is consistent with our results. However, Aslan and Bayhan [15] reported a significant decrease in CT after refractive correction and patch occlusion treatment for amblyopia. Meanwhile, according to Nishi et al [16], who studied the effect of refractive correction on CT in patients with anisohypermetropic amblyopia, the subfoveal CT in eyes with thicker choroid tended to decrease, while those with thinner choroid tended to increase in both the amblyopic and fellow eyes after wearing corrective lenses. However, they did

Table 3. Comparison of the retinal and choroidal vasculature before and after patch occlusion treatment

	Before occlusion treatment	After occlusion treatment	*p*-value
Width of SFAZ (μm^2, N = 19)	283,271.47 ± 889,934.52	268,690.79 ± 106,569.04	0.437[a]
Width of DFAZ (μm^2, N = 19)	372,730.26 ± 118,685.96	372,670.00 ± 141,447.54	0.998[a]
1-mm fovea SCPD (%, N = 21)	24.18 ± 7.16	22.61 ± 5.70	0.387[a]
3-mm parafovea SCPD (%, N = 21)	50.35 ± 2.22	50.35 ± 2.10	0.289[b]
1-mm fovea DCPD (%, N = 21)	19.44 ± 8.25	18.23 ± 6.56	0.532[a]
3-mm parafovea DCPD (%, N = 21)	54.65 ± 2.01	54.63 ± 2.76	0.973[a]
CT (μm, N = 20)	388.80 ± 105.27	381.63 ± 76.61	0.590[a]
CVI (%, N = 20)	64.49 ± 2.42	62.31 ± 2.50	**0.011**[a]*

Values are presented as mean ± standard deviation or number. Bold text indicates a statistically significant difference. SFAZ, superficial foveal avascular zone; DFAZ, deep foveal avascular zone; SCPD, superficial capillary plexus density; DCPD, deep capillary plexus density; CT, choroidal thickness; CVI, choroidal vascularity index. p-values were calculated using [a]paired t-test and [b]Wilcoxon Signed-Rank test. *$p < 0.05$.

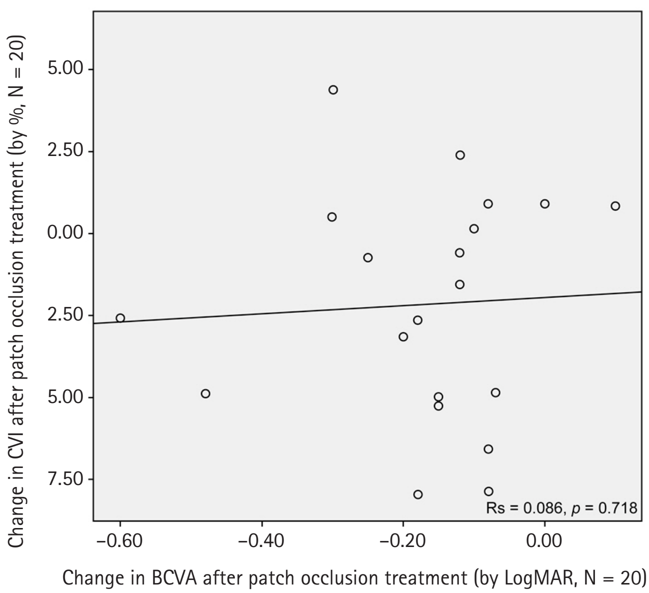

Fig. 5. Scatter diagram showing changes in choroidal vascularity index (CVI) and changes in best-corrected visual acuity (BCVA) of amblyopic eyes before and after patch occlusion treatment. Scatter diagram shows no statistically significant correlation between the two. "Rs" stands for correlation coefficient. Statistical significance was calculated by Spearman's correlation analysis. LogMAR, logarithm of the minimum angle of resolution units.

not confirm the effect of patch occlusion treatment.

There were two studies on the change in choroidal blood flow corresponding to CVI. Nishi et al [17] concluded that luminal/ stromal ratios in the amblyopic eye decreased with refractive correction, becoming closer to the normal control. This is consistent with our findings. They suggested that the optical correction probably led to compensatory changes in the subfoveal choroidal microvasculature in the amblyopic eye. But since our study considers the effect of patch occlusion treatment, we sought other explanations for our results. Hashimoto et al [18] reported that in two cases of anisohypermetropic amblyopia, choroidal blood flow which corresponding to CVI in our study increased after refractive correction and patch occlusion treatment. Our results did not confirm this finding.

Meanwhile, a recent study [19] found that the SCPD and DCPD were lower in the amblyopic eyes of untreated patients than in healthy controls, but the recovered amblyopic eyes after refractive correction and patch occlusion treatment did not differ significantly from the healthy control eyes, and there was no difference in the macular thickness among the three groups. This is inconsistent with our study, since we found no significant difference between SCPD and DCPD before and after patch occlusion treatment in the amblyopic eye.

Based on previous studies [20-26] related to amblyopia, we hypothesized that if there was a difference in retinal and choroidal vasculature between the amblyopic eye and the normal eye, changes in the vasculature of the amblyopic eye may occur after patch occlusion treatment. The data showed statistically significant differences in CVI before and after the treatment. According to several studies, CVI in the amblyopic eye is thought to be higher than that in the normal or fellow eye [27-30]. In our study, CVI may have decreased because of an increase in the choroidal stroma in the amblyopic eye due to patch occlusion treatment for the fellow eye. According to Terada et al [30], the amblyopic eye was found to have an increased vascular area in the outer choroid, relative to that in the normal control group. Nishi et al [29] found a significantly larger luminal area and a significantly smaller stromal area in the amblyopic eyes than in the control hyperopic eyes. Both studies confirmed that the choroidal stroma in the amblyopic eye was lower than that of the normal eye and demonstrated that this influenced the development of amblyopia. Patch occlusion treatment increases the visual stimulation of the amblyopic eye and develops the visual function [2]. Although the process is not clear, the increased visual stimulation of the amblyopic eye through patching may have led to an increase in the choroidal stroma, resulting in a decrease in CVI in the amblyopic eye.

Second, CVI may have decreased due to constriction of the choroidal vessels of the amblyopic eye following patch occlusion treatment. Borrelli et al [28] found an increase in the choriocapillary vessel density in the amblyopic eye, relative to that in the normal control eye. Baek et al [27] reported a higher CT and choroidal vasculature in amblyopic eyes than in control eyes. Both studies suggested that the increased CVI in the amblyopic eye is due to the increased blood flow needed to meet the requirement of the retina. As the visual function of the amblyopic eye improved by patch occlusion treatment, the blood flow requirement of the retina may have decreased; thus, the choroidal vessels may have constricted. CVI is the ratio of the vascular area to the entire choroidal area; therefore, the CVI decreases. Here, since there was no significant difference in CT before and after the treatment, the two phenomena suggested above may have occurred together. However, to prove this hypothesis, a prospective follow-up study with a larger number of patients is warranted.

Conversely, the degree of decrease in CVI and the degree of improvement in BCVA were not correlated with each other in this study, with the reason being unclear. During patch occlusion treatment, visual stimulation of the amblyopic eye increases and the choroidal blood vessels constrict, but the recovery of vision is known to be related to the development of both the retina and the visual cortex [2-4]. During the treatment of amblyopia, changes in the visual cortex may have a greater effect on the improvement of visual acuity than structural changes in the ocular microvasculature and thus the decrease in CVI is not necessarily correlated with changes in VA. However, an adequate number of patients, divided by the follow-up period, should provide more accurate results for the correlation between changes in the CVI and BCVA.

There were several limitations to this study. First, this was a retrospective study, and with the limited sample of 17 patients with 26 eyes, and without any control group, it would be difficult to apply the results to the entire population. Second, the daily treatment time and follow-up period varied, and the effect of these differences on the outcome could not be confirmed. Third, since axial length was not measured in this study, it was not taken into account as a confounding factor. Finally, differences in treatment between binocular amblyopia and monocular amblyopia were not considered. Therefore, a prospective and follow-up study of a large number of patients is required to compensate for these limitations.

In conclusion, a decrease in CVI was observed after patch occlusion treatment, although a relationship between CVI and BCVA could not be established. A decrease in CVI implied an anatomical change in the choroid. Therefore, patch occlusion treatment in amblyopia appears to affect the changes in the choroidal vessels and stroma. Future studies are required to confirm our results and to determine the relationship of retinal and choroidal changes with changes in BCVA.

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Conflict of interest

The authors declare no conflicts-of-interest related to this article.

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