Role of the Choroidal Vascularity Index in Branch Retinal Vein Occlusion with Macular Edema

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Abstract

The purpose of this study was to assess choroidal vasculature changes in eyes with branch retinal vein occlusion (BRVO) and macular edema (ME) using the choroidal vascularity index (CVI) and evaluate the effectiveness of CVI as a prognostic biomarker. 35 patients (70 eyes) with BRVO and ME were analyzed retrospectively. Luminal and stromal areas in choroids of swept-source optical coherence tomography were calculated using the image binarization technique. The CVI was calculated as the ratio of the luminal to total choroidal area. The CVI of BRVO and ME eyes were compared with that of the unaffected fellow and post anti-vascular endothelial growth factor (VEGF) injected eyes. A regression analysis was performed on the choroidal parameters and logMAR visual acuity (VA) two years post disease onset. The CVI of BRVO and ME eyes was significantly lower than the fellow and post-injected eyes ($p < 0.05$). The regression analysis showed a strong association between two years after logMAR VA and the CVI of fellow eyes ($R^2 = 0.433$, $p < 0.001$). No remarkable R^2 values were observed in the CVI and subfoveal choroidal thickness of BRVO and ME eyes ($R^2 = 0.189$, 0.155 , respectively, $p < 0.05$). Reduced CVI in BRVO and ME suggests that retinal ischemia and choroidal vascular changes might be closely related. The fellow eye CVI could be a useful supplementary prognostic biomarker.

Introduction

Branch retinal vein occlusion (BRVO) is a mechanical vascular obstructive disease characterized by retinal hemorrhage, macular edema (ME), or neovascularization.¹ Decreased visual acuity (VA) mainly occurs due to ME depending on the degree of the ischemic condition. The pathogenesis of ME in BRVO has been shown to be due to the increased concentration of vascular endothelial growth factor (VEGF) in retinal ischemia altering the inner blood-retina barrier (BRB) structure, causing flood shift from the vessel components to the retinal cellular components.² As sustained macular edema can result in permanent visual disturbance, clinicians should ensure timely treatment with intraocular anti-VEGFs or steroids.^{3,4}

Besides, the advent of swept-source optical coherence tomography (SS-OCT) and enhanced depth imaging spectral-domain OCT (EDI-OCT) has enabled ophthalmologists to perform quantitative measurements of the choroidal area, resulting in increased interest in the relationship between retinal vascular disease and choroid vascular structure.^{5,6}

Several studies have found an increase in the subfoveal choroidal thickness (SFCT) of patients with BRVO that tends to decrease after anti-VEGF, or dexamethasone treatment.⁷⁻¹² This implies that the effects of retinal VEGF are not just confined to the retina, but also reach the choroidal structure beyond the barrier of retinal pigment epithelium (RPE) tight-junction. Although we could assume that choroidal vascularity reflects the severity of retinal diseases, there has been much debate regarding the suitability of using SFCT as a parameter to predict prognosis as SFCT varies due to age, gender, refractive errors and other factors.¹³ However, a novel, more accurate choroidal structural analysis, called the choroid

vascularity index (CVI) has now been introduced. This enables the ratio of the luminal area (LA) to the stromal area (SA) to be calculated by image binarization.^{13–15}

In the current study, we identified how the choroidal vasculature changed in eyes with BRVO and ME, by comparing diseased eyes with unaffected fellow and anti-VEGF treated eyes using the CVI. Furthermore, we performed several analyses to ascertain whether there were any correlations between the later VAs and choroidal measures (including unaffected fellow eyes) to assess the effectiveness of using CVI as a prognostic biomarker.

Methods

Study Population

This retrospective observational study was conducted in the Department of Ophthalmology and Visual Science in Seoul St Mary's Hospital and adhered to the tenets of the Declaration of Helsinki. All protocols were approved by the Institutional Review Board of Seoul St. Mary's Hospital, The Catholic University of Korea Catholic Medical Center, South Korea. All patients included in this study provided verbal Informed consent. Owing to the retrospective nature and anonymized data of this study, the written informed consent procedures had been exempted under the provisions of the Institutional Review Board of Seoul St. Mary's Hospital (KC20RISI0986).

Diseased eyes of patients diagnosed with monocular BRVO and ME at our clinic and their corresponding fellow eyes as controls were used. All participants were recruited between June 2017 and February 2020 at Seoul St. Mary's Hospital in Korea, and a retrospective review of their medical records was performed. The exclusion criteria were as follows: (1) refractive errors of more than $\pm$ six diopters (as spherical equivalent), (2) eyes with a history of any ocular trauma, laser treatment, or intraocular surgery, (3) eyes with a history of intravitreal injections, (4) other systemic diseases that could affect the eye, including diabetes mellitus, (5) presence of other retinal diseases, including glaucoma, age-related macular degeneration, diabetic retinopathy, pachychoroid disease, or neurodegenerative disease (6) media opacity that could affect image quality, (7) any history of uveitis.

Study Protocol

Demographic data, medical history, and ophthalmologic history were recorded at the initial visit. All subjects underwent an ocular examination, which included a best-corrected visual acuity (BCVA) evaluation, non-contact pneumatic tonometry, slit-lamp microscopy, dilated fundus examination, and OCT. The initial and two year VA using the Snellen chart were converted to the logarithm of the minimum angle of resolution scale (logMAR). Imaging was obtained with an SS-OCT device (DRI Triton, Topcon, Tokyo, Japan) using a 1050-nm wavelength light source, and a scanning speed of 100,000 A-scans/second. A 6-line radial pattern scan (1024 A-scans) centered on the fovea was performed for each eye.

Image Analysis

BRVO and ME was diagnosed when its typical characteristics were present in a fundus examination and the central macular thickness (CMT) was $> 300 \mu\text{m}$. CMT was determined using a thickness map in the SS-OCT software. BRVO eyes with superficial hemorrhage involving central macular lesion which could considerably affect the CVI calculation were excluded from image analysis. To minimize the effect of disease periods on the CVI, we analyzed the OCT images of both eyes at the initial visit and the diseased eyes around two months after the first anti-VEGF treatment. All of the OCT images were evaluated by two experienced independent retinal specialists (Y-H.P. and M.K.) who were blinded to the other imaging findings and the patients' clinical histories.

Choroidal Thickness Measurement

Choroidal thickness was calculated using an automatic built-in software within the SS-OCT device. SFCT was determined by calculating the distance from the outer border of the RPE to the inner edge of the suprachoroidal space.¹⁶ We measured SFCT manually at the foveal center using digital calipers provided by the SS-OCT software. Two experienced independent observers measured the SFCT, and the average value was utilized in the analysis to avoid inter-observer variation.

Choroidal Vascularity Index Assessment

A 12 mm raster scan passing through the fovea was chosen for image binarization to obtain the CVI. It was segmented using the protocol described by Agrawal et al.¹³, and image binarization was performed using Image J software (version 1.51; <https://imagej.nih.gov/ij/>). With the polygon selection tool, the total choroidal area (TCA) was selected, and regions of interest (ROIs) were added to the ROI manager (Fig. 1A). After converting the image into 8 bit, a Niblack auto local threshold¹⁷ tool was applied, which gave the mean pixel value with the standard deviation (SD) for all the points. After using the color threshold tool, the SA was highlighted and subsequently added to the ROI manager. Both of the initially selected polygonal TCA and highlighted SA were selected and merged through an "AND" operation in the ROI manager. This composition of areas was added to the ROIs manager as a third area. The LA in the polygon was determined by subtracting the third composite area (SA) from the total polygon area (TCA; Fig. 1B). The ratio of LA to TCA was defined as the CVI.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences for Windows version 22.0 (SPSS, Inc, Chicago, IL). An exploratory analysis was conducted for all variables. The mean differences between the diseased, fellow, and anti-VEGF treated eyes were assessed using the Wilcoxon matched-pairs signed-rank test. A linear regression analysis was conducted between the choroidal parameters and logMAR VA measured two years post the onset of the disease. A Mann-Whitney U test was used to compare the choroidal measurements in the two sub-groups divided by the degree of CMT at the initial visit. Univariate and multiple linear regression analyses were performed to analyze the effects of multiple factors associated with the CVI. Two-sided p-values of < 0.05 were considered to be statistically significant.

Results

This study included 35 diseased and 35 unaffected contralateral (fellow) eyes from 35 patients diagnosed with monocular BRVO and ME at our clinic. Their 35 unaffected eyes were used as controls. Demographics and characteristics, including the macular and choroidal measurements (CMT, CVI, SFCT), are presented in Table 1. The mean CVI of the BRVO eyes was $62.16 \pm 2.08\%$, which was significantly lower than that of the fellow ($p < 0.001$) and post-injected BRVO eyes ($p = 0.025$). The mean SFCT of the BRVO eyes was $253.62 \pm 66.61 \mu\text{m}$, which was not significantly different when compared to the fellow eyes ($p = 0.447$), but significantly higher than post-injected BRVO eyes ($p = 0.008$; Fig. 2).

Table 1
Demographics, characteristics and choroidal measurements of the study subjects (n = 35). Data are presented as the mean ($\pm$ SD) or a number (%), as appropriate. BCVA: Best-corrected visual acuity; IOP: intraocular pressure; CMT: central macular thickness; CVI: choroidal vascularity index; SFCT: subfoveal choroidal thickness.

	Total n = 35
Age, years	65.85 ($\pm$ 11.53)
Sex, male:female	16:17
Hypertension, n (%)	10 (28.6%)
Disease eye, OD:OS	18:17
Initial BCVA, decimal	0.40 ($\pm$ 0.24)
Initial IOP, mmHg	14.02 ($\pm$ 3.28)
Refraction, spherical equivalent	-0.82 ($\pm$ 2.73)
Initial CMT (μm)	494.34 ($\pm$ 134.67)
CVI (%)	62.16 ($\pm$ 2.08)
Fellow eye CVI (%)	63.71 ($\pm$ 3.34)
Post-injected CVI (%)	63.01 ($\pm$ 2.34)
SFCT (μm)	253.62 ($\pm$ 66.61)
Fellow eye SFCT (μm)	246.31 ($\pm$ 76.96)
Post-injected SFCT (μm)	234.28 ($\pm$ 68.80)

The results of the linear regression analysis between the possible prognostic choroidal values and logMAR VA two years post disease onset are shown in Fig. 3. The CVI of the unaffected fellow eyes revealed the most prominent R^2 value (0.433) and significant p-value ($p < 0.001$; Fig. 3B). However, no

remarkable R^2 values were found in the CVI or the SFCT of BRVO eyes (0.189, 0.155, respectively) despite significant p-values (0.009, 0.019, respectively, Fig. 3A, 3C).

A sub-group analysis was performed to comprehend which choroid parameter most accurately reflected the severity of macular edema. Eighteen patients were assigned to the severe CMT group, revealing CMT over 500 μm at their initial visit, which was the median value of all the subjects. Seventeen patients were assigned to the moderate CMT group (CMT lower than 500 μm). The CVI of the fellow eyes was significantly lower in the severe group than the moderate group ($p = 0.013$). No prominent p-values were observed in any of the other choroidal measurements (Table 2).

Table 2

Sub-groups analysis using Mann-Whitney U Test for the choroidal parameters between the severe central macular thickness (CMT) group (CMT > 500 μm) and moderate CMT group (CMT < 500 μm) at the initial visit. Data are presented as the mean ($\pm$ SD). p-values that are statistically significant are highlighted in bold. ME: macular edema; CVI: choroidal vascularity index; SFCT: subfoveal choroidal thickness.

	Severe ME (N = 18)	Moderate ME (N = 17)	p-value
CVI (%)	61.61 $\pm$ 1.48	62.73 $\pm$ 2.48	0.118
Unaffected fellow eye CVI (%)	62.60 $\pm$ 2.29	64.89 $\pm$ 3.91	0.013
SFCT (μm)	265.38 $\pm$ 63.18	241.17 $\pm$ 69.77	0.424
Unaffected fellow eye SFCT (μm)	254.16 $\pm$ 80.60	238.00 $\pm$ 74.44	0.782

A univariate analysis was performed to evaluate the factors that can affect the CVI values. The univariate regression analysis revealed that age, sex, BCVA, hypertension, intraocular pressure (IOP), and SFCT were not significantly associated with the CVI ($p > 0.05$), except for CMT ($p < 0.05$). A multivariate linear regression analysis of factors with a p-value lower than 0.2 in the univariate analysis showed that no variables significantly correlated with the CVI (Table 3).

Table 3

Linear regression analysis of the factors associated with the CVI. ^aAdjusted for variables with a P value < 0.20 in the univariate analysis. β , regression coefficient. p-values that were statistically significant are highlighted in bold. BCVA: Best-corrected visual acuity; logMAR: logarithm of the minimum angle of resolution; IOP: intraocular pressure; CMT: central macular thickness; SFCT: subfoveal choroidal thickness.

	Univariate		Multivariate ^a	
	Standardize β	p-value	Standardize β	p-value
Age, years	0.048	0.139	0.022	0.501
Sex, female	0.192	0.799		
BCVA, logMAR	-0.837	0.452		
Systemic hypertension	0.077	0.923		
IOP, mmHg	-0.171	0.119	-0.169	0.107
CMT, μm	-0.006	0.033	-0.005	0.112
SFCT, μm	0.005	0.332		

Discussion

In this study, one possible mechanism of the decreased CVI in the eyes with BRVO and ME eyes could be VEGF-related choriocapillaris (CC) regression. Despite our efforts to exclude choroidal changes on disease duration in BRVO and ME by collecting OCT scans at the initial visit, there may be some temporal gaps between the time when an obstruction with ischemia began and when the OCT scan was initially performed. The CVI values could be measured higher if the OCT scans are taken when the obstructions first occur, or when the VEGF concentrations are not elevated enough to cause degeneration of the CC. The role of VEGF as a vasodilator in choroids could be inversely inferred from previous reports that anti-VEGFs act as vasoconstrictors for choroidal supply for one week after the injection.^{18,19} Therefore, we presume that when exposed to excessive VEGFs over time such as in BRVO and ME, the CC would undergo some structural changes like microaneurysms or constrictions, leading to capillary blockage from the altered hemodynamics. Tee et al.²⁰ reported that the CC diameters, number of CC lacunae, and choroidal arterioles/venules significantly decreased in the retinal VEGF overexpressed transgenic mice model after only a few weeks. As the structural damages were prominent in small vessels, especially CC in that study, it would make sense to regard CC regression as the main reason for decreased CVI in our study rather than large choroidal vessel collapse. Even if CC was too thin layer to have a significant effect on CVI by itself, CC regression would increase the resistance of the overall choroidal vessels, resulting in decreased choroidal blood flow. Aribas et al.²¹ considered a pressure effect of choroidal congestion as the main cause of decreased CVI and CC flow density in BRVO. However, the supposition differs from ours in that the gradual effect of excessive VEGF on the choroid vascular structure was totally excluded

in the report. Of course, the fact that the population of their study was stable patients without ME would contribute a disagreement on the underlying reason of CVI reduction in BRVO ME. Further researches are needed to elucidate a more robust theory of decreased CVI and CC flow reduction in the retinal ischemic condition.

Several optical coherence tomography angiography (OCTA) studies have shown that CC density and flow decreased in RVO and ME and improved after anti-VEGF, or dexamethasone were injected.^{22,23} A second possible mechanism, as these studies mentioned, could be a shadow effect of the overlapping ME. The two studies above suggested that ME-induced signal attenuation of choroids in SD-OCT results in decreased CC parameters. In our study, the univariate regression analysis showed that only CMT was significantly associated with CVI, supporting the possibility that ME-induced choroidal signal attenuation or mechanical pressure somewhat influenced the calculation of CVI. However, CC regression by lasting BRVO and ME should be highlighted, in regards of decreased CVI because our study used an SS-OCT device, which helps to clearly visualize the choroid by having a longer center wavelength and minimizing RPE-induced signal attenuation.

The increased CVI after anti-VEGF treatment in our study could also be explained by the recovered signal attenuation of choroids as ME decreased. However, we propose that the high concentration of VEGF could trigger an increase of choroidal blood flow two months after the anti-VEGF treatment. Okamoto et al.²⁴ found that in patients with recurrent BRVO and ME, defined as macular thickness over 250 μm after anti-VEGF treatment, choroidal blood flow measured by laser speckle flowgraphy decreased significantly one week after the injection, but increased over time. As 29 patients in our study almost met the criteria of that recurrent group and all of them experienced at least one recurrent episode, our results could be due to more VEGFs impacting on the vasodilatory function in choroids two months after the injection. One possible hypothesis is that retinal VEGF and anti-VEGF compounds could alter RPE permeability, increasing the retina to choroid VEGF gradient. The influx of retinal VEGF to the choroid would increase the choroidal blood flow, resulting in an increased CVI after the anti-VEGF injection. Campa²⁵ reported that the coadministration of anti-VEGFs with VEGF121 or VEGF165 resulted in a marked increase in macromolecular permeability. The second hypothesis is that hypoxia-induced RPE-origin VEGFs, discussed in a report by Arjamaa et al.²⁶, accumulate in choroids over time, even though anti-VEGFs reduce active retinal VEGFs and ME. Some studies have demonstrated that even with consecutive anti-VEGF treatments, retinal ischemia could still be aggravated in patients with central RVO (CRVO).^{27,28} Therefore, unresolved hypoxia could lead to a high production of RPE-origin VEGF, and this newly produced VEGF might affect the choroid more than the retina two months after treatment.

The SFCT values in our study were only slightly consistent with that of previous studies in that no significant change was found between the BRVO and fellow eyes, but SFCT decreased after anti-VEGF treatment. The result of increased CVI with decreased SFCT after anti-VEGF injection could be explained by fluid shift between choroidal vessel and stroma. In condition of increased vascular resistance due to CC regression, increased choroidal inflow by VEGF would elevate hydrostatic pressure, leading to fluid

shift toward stroma. In the contrary, reduced hydrostatic pressure by anti-VEGF injection could be advantageous to maintain intravascular volume, showing the result of increased CVI with decreased SFCT. We also speculate that several factors, including IOP or systemic blood pressure fluctuations, could have affected the SFCT values in our study.¹³ The exact region of the retinal vessel obstruction could also affect the SFCT.²⁹

For clinical application, we performed a linear regression analysis between the choroidal parameters and logMAR VA two years post disease onset. Although the exact locations of the vein obstruction varied in BRVO, and the degree of ischemia were not necessarily consistent with the final VA, the prognosis of BRVO and ME must be related to the VA after treatment no matter how much recurrence occurs. As the CVI of the contralateral fellow eyes showed the most prominent values among the coefficients of determination, inherent choroidal vascularity could be considered as a buffer system in response to retinal ischemia. Patients with a higher vessel proportion in the choroid were more likely to have an abundant CC, which would supply oxygen and nutrients effectively to the outer retina even in the process of CC regression over the course of BRVO and ME. Khodabandeh et al.³⁰ also reported that visual outcomes were significantly associated with OCTA CC flow in patients with CRVO.

In the additional sub-groups analysis, the most significant correlation between fellow eye CVIs and the degree of ME agreed with the hypothesis that the inherent choroidal vessel portion, the fellow eye CVI, affected the formation of the retina to the choroid VEGF gradient discussed earlier. Some articles have suggested a compartment effect.^{22,31} This means that if individuals originally had a large amount of vascular component in their choroids, the choroidal VEGF sensitivity might be much higher than in the lower groups, possibly reducing the severity of ME.

There were some limitations to this study. A small number of patients were included in the analysis, and only a single macular scan was used to calculate the CVI. A 3D volume image could be measured using OCTA, which would enable the choroidal vessel components to be calculated as a volume rather than an area.³² Mehta et al.³³ introduced methods of OCTA image binarization thresholding and brightness/contrast adjustment. If combined with the new modalities that have enabled ophthalmologists to measure choroidal deep vessel structures beyond the CC layers, OCTA could help to provide more accurate information, such as a volumetric CVI. In addition, as the CVI was manually calculated in our study, newly invented automatic calculation software may help to improve the reproducibility of the CVI measurements. To include a wide range of the ischemic area, we selected the whole choroidal area on the macular scan. However, it might be more useful only to measure the foveal lesions. Further studies should explore a more robust method.

This study revealed the association between BRVO and CVI with SS-OCT for the first time. As it has prognostic value, the concept of using the CVI of the unaffected fellow eye could help to promote the discovery of more elaborate indexes or biomarkers for clinical application, along with the development of new modalities. Considering the choroid vascular component as one of the crucial elements in

determining the final visual outcome in retinal vascular disease, we have shown that choroidal measurements could considerably support retinal biomarkers in BRVO and ME.

In conclusion, the reduced CVI in eyes with BRVO and ME suggests that retinal VEGF and ischemic condition may affect choroidal vascularity by altering the CC structure. The CVI of the unaffected fellow eye could be a useful supplementary prognostic biomarker in patients with monocular BRVO and ME.

Declarations

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Author Contributions

Bo-Een Hwang (B.H.), Mirinae Kim (M.K.), Young-Hoon Park (Y.H.P.). Drafting/revising the manuscript for content, including medical writing for content: B.H., M.K., Y.H.P. Study concept or design: B.H., Y.H.P. Analysis or interpretation of data: B.H., M.K., Y.H.P. Acquisition of data: B.H. Statistical analysis: B.H. Study supervision or coordination: B.H., M.K., Y.H.P.

Additional Information

Competing interests: None of the authors have any conflicting interests to disclose.

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Figures

Figure 1

The choroidal vascularity index (CVI) measuring process with swept-source optical coherence tomography. The total choroidal area (TCA) was determined using the polygon selection tool in ImageJ software (A). Following image binarization using the Niblack auto local threshold tool, the stromal area (SA) within the selected TCA was highlighted. A distinction was set between the SA (the yellow part) and the luminal area (LA; the black part) (B). The CVI was calculated as the ratio of LA to TCA.

Figure 2

The choroidal vascularity index (CVI) and subfoveal choroidal thickness (SFCT) were measured to compare the branch retinal vein occlusion (BRVO), unaffected fellow, and post anti-vascular endothelial growth factor (VEGF) injected BRVO eyes. (A) The CVI of the BRVO eyes was significantly decreased compared to the unaffected fellow and post-injected eyes (all $p < 0.05$). (B) The SFCT of the post anti-VEGF injected eyes was significantly decreased compared to the BRVO eyes ($p < 0.05$), with no significant

change between the BRVO and unaffected fellow eyes found ($p=0.447$; Wilcoxon matched-pairs signed-rank test).

Figure 3

The linear regression analysis between the choroidal parameters and logarithm of the minimum angle of resolution scale (logMAR) visual acuity measured two years post the disease onset. The R^2 and p-values are presented. (B) The choroidal vascularity index (CVI) of the unaffected fellow eyes reveal the most prominent R^2 value and a significant p-value; (A),(C) Despite the significant p-values, no remarkable R^2 values are presented in the CVI and subfoveal choroidal thickness (SFCT) of the eyes with branch retinal vein occlusion (BRVO).