Inner Retinal Microvasculature Damage Correlates With Outer Retinal Disruption During Remission in Behçet’s Posterior Uveitis by Optical Coherence Tomography Angiography

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PURPOSE. To quantify the inner retinal vascular changes that occur in the superficial and deep layers in patients with Behçet’s disease (BD) in remission using optical coherence tomography angiography (OCTA) and to evaluate the associations with outer retinal structure.

METHODS. Nineteen eyes from 19 patients with BD in remission were enrolled, including 10 eyes with less than five ocular attacks (n < 5) and nine eyes with five or more attacks (n ≥ 5). The foveal avascular zone (FAZ) and global and regional vessel density (VD) in both layers were compared between BD eyes and normal eyes. Their outer retinal structure, including integrity of the ellipsoid zone (EZ), interdigitation zone (IZ), and outer retinal layer thickness were evaluated. Associations between the inner retinal vasculature and outer retinal disruption were sought.

RESULTS. Compared to normal eyes, except for the nasal region, all deep capillary VD values were lower in the BD groups, especially in the inferior region. In the superficial layer, the VD differences between groups were larger in capillaries than in small vessels. The FAZ in the n ≥ 5 group was larger than that in normal and the n < 5 groups in the deep layer. Greater disruption of EZ and IZ was correlated with decreasing global and regional deep capillary VD.

CONCLUSIONS. BD Patients in remission had significant changes in the inner retinal vasculature that corresponded to the outer retinal disruption. Quantitative measurement by OCTA and algorithm might be useful for evaluation of the vasculature and pathologic changes in BD.

Keywords: retinal microvasculature, Behçet disease, optical coherence tomography angiography, retinal structure

Behçet’s disease (BD) is a chronic, relapsing, multisystemic immune-mediated vasculitis of unknown etiology. Ocular involvement is characterized by recurrent nongranulomatous uveitis with necrotizing obliterator retinal vasculitis that may lead to blindness. Progressive retinal vasculitis and resulting retinal damage are features of posterior segment involvement, so spectral-domain optical coherence tomography (SD-OCT) has become important for evaluation, diagnosis, and monitoring of BD. Moreover, OCT angiography (OCTA) provides high-resolution, depth-resolved images for evaluation of various ophthalmic diseases, including diabetic retinopathy, artery or vein occlusion, and uveitis in a rapid, noninvasive, dye-free manner.

The oxygen demand in the avascular outer retina in humans depends primarily on diffusion from the choroidal circulation. However, a recent study demonstrated that the deep capillary plexus of the inner retinal vascular layer contributes about 15% of the oxygen requirements of the photoreceptor inner segment. This contribution becomes even more significant in the setting of hypoxia, where the choroidal vasculature cannot autoregulate its blood supply. Choroidal involvement and dysfunction of the vasculature in BD has been suggested by histopathologic and in vivo clinical studies. Disorders of the outer retina and progressive damage to photoreceptors have also been demonstrated in BD. These findings indicate that the outer retinal disruption and choroidal disorders in BD may be related to changes in the inner retinal vasculature.

OCTA with split-spectrum amplitude-decorrelation angiography can analyze the layers of the retinal microvasculature with high intravisit repeatability and intervisit reproducibility. Previous SD-OCT studies have revealed an association of dysfunction in the deep retinal capillaries with disruption of the outer retina in diabetic retinopathy with diabetic choroidopathy. Furthermore, our previous work has quantified the intraretinal changes around the macula in BD and reported significant disruption of the outer retinal structures during remission.

Knowledge concerning the changes that occur in the layers of the inner retinal vessels in BD is limited. Previous studies using fluorescein angiography (FA) could not provide sufficient high-resolution detail in the retinal capillaries for differential layer analysis because of technical limitations. Two studies using OCTA described the perifoveal microvascular changes in
BD that occur during the active phase.\textsuperscript{6,23} However, no study has quantified the microvasculature of the different retinal layers in BD during remission and associated these changes with damage to the outer retina.

The aim of this prospective study was to characterize the retinal vascular plexuses in the superficial and deep layers around the macula in patients with BD in remission using OCTA. We also investigated the association of the microvascular changes in the inner retina with outer retinal damage using SD-OCT.

**METHODS**

**Participants**

This prospective observational study was performed from October 2015 to January 2017. The study protocol was approved by the Ethics Committee at Wenzhou Medical University and complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients and subjects before imaging. The inclusion criteria for the BD groups were fulfillment of the diagnostic criteria developed by the International Study Group for Behçet’s Disease,\textsuperscript{24} BD in remission,\textsuperscript{18} and evidence of posterior segment involvement.\textsuperscript{25,26} Exclusion criteria for eyes with BD were anterior uveitis only, a spherical equivalent refractive error greater than $\pm 6.0$ diopters, significant cataract or vitreous opacity, diabetic retinopathy, age-related macular degeneration, glaucoma, other marked complications, or a history of intraocular surgery within the previous year. We selected the eye with higher signal-strength index in patients with bilateral Behçet’s uveitis. Normal subjects with no history of systemic disease and matched for age, sex, and spherical equivalent were recruited as controls. The right eye of each subject was selected. The exclusion criteria for the control group were ocular disease (e.g., diabetes retinopathy or glaucoma), best-corrected visual acuity (BCVA) $<20/20$, and a spherical equivalent refractive error more than $\pm 6.0$ diopters.

We divided the patients with BD into two groups according to the number of ocular attacks ($n < 5$ or $n \geq 5$). Each patient underwent a complete ocular examination, including slit-lamp examination, intraocular pressure measurement (Goldman applanation tonometry), funduscopy, FA, and OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) and OCTA (AngioVue; Optovue, Inc., Fremont, CA, USA). Demographic and clinical data were collected, including for sex, age, interval between the last ocular attack and the current remission phase, duration of Behçet’s uveitis, and number of attacks. All patients were examined by the same uveitis specialist. Mydriasis was obtained by applying eyedrops containing 0.5% tropicamide and 0.5% phenylephrine hydrochloride to both eyes at least 30 minutes before imaging. The spherical equivalent refractive error of each eye was measured using an autorefractor (KR8900; Topcon, Tokyo, Japan). BCVA was measured using a Snellen chart and converted to logarithm of the minimum angle of resolution (logMAR) units for analysis.

**Image Acquisition and Data Analysis**

All patients with BD and controls underwent SD-OCT imaging performed by a single operator. The central $3 \times 3$-mm area for each eye was analyzed. According to the automated setting, the superficial network extends from $3 \mu m$ below the internal limiting membrane to $15 \mu m$ below the inner plexiform layer. The deep capillary network extends from $15$ to $70 \mu m$ below the inner plexiform layer.\textsuperscript{27} A cut-off signal-strength index value of $\geq 40$ (defined by the machine) was taken as the study inclusion criterion. Poor OCTA images with significant defects\textsuperscript{28} (motion artifacts, projection artifacts, vessel doubling, and/or stretching defects) were excluded. Custom-built imaging software implemented in MATLAB (Mathworks, Natick, MA, USA) was used to separate the foveal capillary vascular ring from the background image using an active contour model based on a level-set method\textsuperscript{29} and automatically

![Figure 1](http://arvojournals.org/)
measured the foveal avascular zone (FAZ) in the superficial and deep networks (Figs. 1B, 1E). For the measurement of FAZ area, 15 normal eyes were assessed for repeatability. Two high-quality images were obtained from each eye. The mean brightness of the central FAZ was measured and taken as the threshold for examination of the superficial and deep retinal layers. A brightness value greater than the threshold was shown as the flow signal and taken to indicate vessels (Figs. 1C, 1F). Vessel density (VD) was automatically measured and calculated as the percentage of pixels with flow signal.

Two concentric circles centered on the fovea, with a diameter of 1 and 3 mm, were made for each eye (Fig. 2A). For the superficial layer, the vessels in the area within the two circles were divided into small vessels (Fig. 2B) and capillaries (Fig. 2C) using the Horton-Strahler scheme. The vessels were graded automatically and visually inspected by two of the authors (DC, XZ). In the area within the two circles that was calculated automatically, the global superficial and deep capillary VD values were defined as the percentage of pixels in the superficial layer and deep layer, respectively (Figs. 3A, 3D). The global superficial small VD was defined as the pixel area of small vessels divided by the area within the two circles (Fig. 3B). For measurement of the global superficial capillary VD, the pixels of the small retinal vessels were dropped from the whole image. The pixel area of the superficial retinal capillaries was then calculated (Fig. 3C). The region between the two concentric circles was divided equally into superior, inferior, temporal, and nasal zones (Figs. 3E–H). Pixels of vessels were calculated in the four separate regions.

For the patients with BD, one cross-sectional OCT image with a quality score $\geq 20$ was selected along the horizontal and vertical meridians passing through the fovea. The intraretinal outer retinal layer (ORL), defined from the inner edge of the outer plexiform layer to the inner boundary of the retinal pigment epithelium, was automatically segmented and checked by the same two authors (DC, XZ) to obtain precise thickness measurements. For each eye, the thickness profiles along the horizontal (Fig. 4C) and vertical (Fig. 4D) scans within the outer diameter of 6 mm were averaged to obtain the mean ORL thickness. The integrity of the ellipsoid zone (EZ) and interdigitation zone (IZ) was evaluated in each image for $500 \mu m$ in either direction of the fovea along the horizontal and

![Figure 2](http://arvojournals.org/) Postprocessed images illustrating the divided retinal vessels. (A) Image containing whole retinal vessels. (B) Image containing only the divided retinal small vessels. (C) Image containing only the divided retinal capillaries.

![Figure 3](http://arvojournals.org/) The macular region analyzed by the automated algorithm for analysis of VD. Two concentric circles were created with diameters of 1 and 3 mm, respectively, centered on the fovea. (A–C) Images for calculation of global superficial VD, superficial small VD, and superficial capillary VD, respectively. (E–G) Images for calculation of regional superficial VD, superficial small VD, and superficial capillary VD, respectively. (D, H) Images used for measurement of global and regional deep capillary VD. S, superior; N, nasal; I, inferior; T, temporal.
vertical scans (Figs. 4C, 4D). The line disruption was graded from 0 to 2. Grade 0 was assigned when the line was intact, and grades 1 and 2 were assigned when there was a focal disruption of the line of $\leq 200 \mu m$ and $>200 \mu m$, respectively. The global disruption scale was the added grades of each patient’s horizontal and vertical scans. The structure of the outer retinal bands was shown by a longitudinal reflectivity profile (LRP; Figs. 4E, 4F). OCT reflectivity was measured for approximately 40 A-scans over 500 $\mu m$ in both directions of the central foveal horizontal and vertical meridians. Finally, the mean LRP was constructed by averaging all the LRPs along both directions.

Statistical Analysis

The data are expressed as the mean $\pm$ standard deviation and were analyzed using statistical software (SPSS version 22.0; IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to verify normality of distribution for continuous variables. To determine statistically significant differences between groups, we used independent $t$-tests for normally distributed continuous variables. The Mann-Whitney $U$ test was used for nonnormally distributed continuous variables. The paired $t$-test or Wilcoxon signed rank test was used to compare differences in VDs. Univariate ANOVA was performed to test differences in FAZ among three groups. The $\chi^2$ test or Fisher’s exact test was used to compare categorical variables. Pearson’s correlation test was used to determine the association between the area of the intraretinal FAZ, VD, ORL thickness, BCVA, and disease duration. The associations between FAZ area, VD, and disruption of EZ or IZ bands were evaluated using the Spearman correlation test. Bonferroni correction was used for multiple comparisons. The intraclass correlation coefficient (ICC) and coefficient of repeatability and reproducibility (CoR) were analyzed to evaluate the reliability. $P$ values of $<0.05$ were considered to be statistically significant.

RESULTS

Demographic Data

Twenty eyes of 20 patients with Behçet’s posterior uveitis were included, and one eye was excluded because of a poor-quality image. Nineteen eyes with a mean disease duration of $2.7 \pm$
2.0 years were retained for analysis. The n < 5 group contained 10 eyes, and the n ≥ 5 group contained nine eyes. Twenty-five eyes from 25 normal subjects were enrolled as the control group. There was no significant difference in age, sex, or spherical equivalents between the control group and the BD group (Table 1); BCVA was significantly worse (P < 0.001) and signal-strength index was lower (P = 0.002) in the BD group. The FAZ and all VDs in both layers were in accord with normal distribution (P > 0.05).

**Intergroup Differences: Inner Retinal Vasculature and Outer Retinal Disruption**

The FAZ and global and regional VD in the superficial and deep retinal vascular plexus were compared between the normal group and BD group (Table 2). BD caused substantial changes in the VD of the deep capillary plexus. Except for the nasal region, all VDs in the deep vascular layer were smaller in eyes with BD than in normal eyes (P < 0.0023, Bonferroni correction). The differences between groups in global and regional superficial capillary VDs (range, 19.12%-27.81%) were more significant than those in superficial small VDs (range, 5.0%-9.71%, P < 0.001). Compared with the differences in global and regional superficial capillary VDs, the differences in deep capillary VDs (range, 28.99%-32.11%) were more significant (P = 0.002). In all retinal vascular plexuses, of all regions the greatest difference in VDs occurred in the inferior region (19.83%, 10.71%, 27.81%, and 32.11% for superficial vessels, superficial small vessels, superficial capillaries, and deep capillaries, respectively). In eyes with BD, the FAZ values

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### Table 1. Characteristics of Patients With Behçet’s Uveitis and the Normal Controls*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal, n = 25</th>
<th>BD, n = 19</th>
<th>P Value</th>
<th>Difference, %</th>
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<tr>
<td>Eyes, n</td>
<td>25</td>
<td>19</td>
<td></td>
<td></td>
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<td>Age, y (range)</td>
<td>38.7 ± 9.5 (25-54)</td>
<td>37.5 ± 6.5 (27-49)</td>
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<td>Sex, male:female</td>
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<td>14:05</td>
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<td>Spherical equivalent, diopter (range)</td>
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<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, y (range)</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval between last active and current remission phase, mo (range)</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Attacks, n</td>
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<td>10</td>
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<td></td>
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<tr>
<td>≥5</td>
<td>9</td>
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<td></td>
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<tr>
<td>BCVA</td>
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<td>Snellen chart (range)</td>
<td>1</td>
<td>0.72 (0.1-1.0)</td>
<td>&lt;0.001</td>
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<td>logMAR</td>
<td>0</td>
<td>0.19 ± 0.24</td>
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<tr>
<td>Signal-strength index (range)</td>
<td>71.4 ± 6.2 (62-88)</td>
<td>63.8 ± 9.8 (45-82)</td>
<td>0.007</td>
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</tr>
</tbody>
</table>

* For comparisons between normal and BD groups, independent *t*-tests or Mann-Whitney *U* tests were performed as appropriate.

† For sex comparisons, the Pearson χ² test was performed as appropriate.
Correlation of Inner Retinal Vasculature With Outer Retinal Disruption, BCVA, and Disease Duration

In BD eyes, decreased global and regional deep capillary VD values were significantly associated with greater EZ (Spearman r range, −0.797 to −0.833, P < 0.0008, Bonferroni correction; Table 4) and IZ band disruption grading scales (Spearman r range, −0.771 to −0.795, P < 0.0008, Bonferroni correction). For superficial vessels, only superior VD was correlated with a greater EZ disruption grading scale (Spearman r = −0.728, P < 0.0008, Bonferroni correction). There was no correlation of ORL thickness with FAZ or VD in the superficial and deep layers. Decreased global and regional deep capillary VD values were correlated with reduced BCVA (Pearson r range, −0.765 to −0.788, all P < 0.0011, Bonferroni correction; Table 5). In both layers, no correlation was found between disease duration and FAZ or VD.

**DISCUSSION**

Using a commercially available OCTA system and automated algorithms, we found significant decreases in deep capillary VD using OCTA. Of all the regions, the inferior regions were the most severely involved for all retinal plexuses. We also confirmed the hypothesis that changes in the inner retinal vasculature were correlated with damage to the outer retina in eyes with BD.

The decreased VD in the inner retinal plexus during remission of Behçet’s uveitis was the most significant OCTA finding in the present study. We speculate this might be attributed to several possible mechanisms. Obliterative and necrotizing vasculitis is the essential histopathologic finding in eyes with BD. Intraretinal and perivascular infiltrates of inflammatory cells, such as lymphocytes and plasma cells, and excessive expression of multiple adhesion molecules on the vascular endothelium are important in the development of retinal vasculitis in patients with active BD. Recurrent ocular attacks lead to inflammatory retinal vessel occlusions and posterior segment disease. Although our patients were in the remission phase and the inflammation was under control, retinal vasculitis may still have been present because of subclinical inflammatory activity, as in a previous study. Imaging studies using FA and indocyanine green angiography have also identified certain clinical features during the relapsing-remitting course of BD. Recently, in a noninvasive OCTA study, Khairallah et al. detected microvascular changes in eyes with active Behçet’s uveitis and reported that the vascular density values were lower than for normal eyes. It was suggested that focal retinal atrophy results from localized defects in the retinal nerve fiber layer (RNFL), which may also contribute to the nonperfused or hypoperfused areas involving both the superficial and deep retinal plexuses along the pathway of the affected retinal fibers. However, no algorithm was used to divide the retinal vessels into small vessels or capillaries in their study. The suggested pathophysiologic changes within the inner retinal vasculature, which are supported by histopathologic and imaging studies, collectively may have accounted for the decreased blood flow in our study. However, it should be noted that compared with control eyes, the signal-strength signal was lower in BD eyes, which may be associated with media opacity. It was expected and might have an effect on VD measurements.

We found that the decreased VD in the inner retinal vasculature was more significant in the deep plexus layer and in the inferior region. Moreover, in the superficial layer, capillary involvement tended to be more severe than that of

**FIGURE 5.** Number of eyes with BD showing different disruption scales of the EZ and IZ bands. More eyes had disruption of the IZ band than of the EZ band.
A similar predominance of smaller VD was seen on OCTA in various retinal vascular diseases, including diabetic retinopathy, retinal vein occlusion, and other ischemic conditions. Repeated ocular attacks may aggravate retinal ischemia.

Correlational analysis revealed that decreases in deep capillary VD were strongly correlated with worse damage to the outer retinal structures, including the EZ and IZ bands. As a compensatory mechanism of the failed choriocapillaris, the deep capillary plexus of the inner retinal vasculature contributes more to the metabolic demands of the outer retina than it would normally. Furthermore, ischemia in the deep capillary plexus contributes to disruption of the outer retina, including disorganization of the EZ and IZ bands. Thus, the disruption of the outer retina seen on SD-OCT correlates strongly with the nonperfusion of the deep capillaries seen on OCTA. A moderate correlation was found between the decreased superficial capillary VD and EZ disruption scale, suggesting that the superficial plexus vasculature may also contribute to the metabolic demands of the outer retina. However, no relationship was found between ORL thickness and the inner vasculature in the present study. We included patients who had Behcet’s uveitis for a short duration (mean, 3.1 years) and were mainly from the BD1 group in our previous study. As a result of the limited disease duration, macroscopic changes of retina atrophy was absent; however, this does not preclude outer retinal abnormalities in Behcet’s uveitis. Moreover, the association between visual acuity and vascular parameters suggests that the inner retinal networks may be useful for predicting visual acuity damage during remission of BD.

It should be noted that the current study has several limitations. First, the small sample size limits our ability to draw definitive conclusions about the quantitative features of the small vessels. The nonperfusion of capillaries in the deep layer seen on OCTA has been reported in various retinal diseases. When compared with the superficial plexus, the deep capillaries may be more prone to ischemia because they are positioned in a watershed-like region and are not directly connected to arterioles. A similar predominance of capillaries in the deep layer of the RNFL, which are located in the thickest portions of the RNFL, which are connected to arterioles. The major blood vessels are found are positioned in a watershed-like region and are not directly connected to arterioles. A similar predominance of macrovascular damage in the inferior region has been reported in glaucoma and is explained by the association with the RNFL bundles entering the macula vulnerability zone of the disc. Our study also suggests that evaluation of the vasculature in the inferior macula is a useful way of assessing vessel damage in BD. Furthermore, FA studies in patients with posterior Behcet’s uveitis have shown that the retinal capillaries are the most frequently involved vessels, followed by the retinal veins. Although we cannot confirm that inflammatory cells infiltrate the retinal capillaries more often than the retinal vessels, our results nonetheless suggest that OCTA imaging of the inner retinal vasculature, especially the deep retinal capillaries in the inferior region, may be a useful and objective way of assessing involvement of the vasculature during remission of BD. A sensitive evaluation of the retinal plexus may help clinicians to assess the response to treatment and improve monitoring during follow-up.

The EZ was larger in the BD subgroup that had experienced more ocular attacks than both the normal group and the BD subgroup of fewer attacks in the deep layer. Enlargement of the EZ has been reported in various retinal vascular diseases, including diabetic retinopathy, retinal vein occlusion, and other ischemic conditions. Repeated ocular attacks may aggravate retinal ischemia.

Correlational analysis revealed that decreases in deep capillary VD were strongly correlated with worse damage to the outer retinal structures, including the EZ and IZ bands. As a compensatory mechanism of the failed choriocapillaris, the deep capillary plexus of the inner retinal vasculature contributes more to the metabolic demands of the outer retina than it would normally. Furthermore, ischemia in the deep plexus contributes to disruption of the outer retina, including disorganization of the EZ and IZ bands. Thus, the disruption of the outer retina seen on SD-OCT correlates strongly with the nonperfusion of the deep capillaries seen on OCTA. A moderate correlation was found between the decreased superficial capillary VD and EZ disruption scale, suggesting that the superficial plexus vasculature may also contribute to the metabolic demands of the outer retina. However, no relationship was found between ORL thickness and the inner vasculature in the present study. We included patients who had Behcet’s uveitis for a short duration (mean, 3.1 years) and were mainly from the BD1 group in our previous study. As a result of the limited disease duration, macroscopic changes of retina atrophy was absent; however, this does not preclude outer retinal abnormalities in Behcet’s uveitis. Moreover, the association between visual acuity and vascular parameters suggests that the inner retinal networks may be useful for predicting visual acuity damage during remission of BD.

It should be noted that the current study has several limitations. First, the small sample size limits our ability to draw definitive conclusions about the quantitative features of the
inner retinal vasculature and their correlation with pathologic changes in the outer retina. Second, eyes with BD had a lower signal-strength score than normal eyes, which may lead to variations in measurements of vascular parameters. For this reason, we excluded the OCTA image of eyes with a signal-strength index lower than 40. Moreover, the automated algorithm takes the mean brightness of the central FAZ area as the threshold, so we were able to decrease the impact of tissue reflectance on quantification of VD by OCTA. Third, in the OCTA system, projection artifacts have been reported to artificially increase measurements of flow in the deep vascular network. However, a significant decrease in the flow measured in the deep plexus was demonstrated in eyes with BD in the present study. Moreover, upon visual inspection, we excluded images of the deep capillary plexus with significant artifacts. Furthermore, upon visual inspection, we excluded images of the deep capillary plexus with significant artifacts. Third, in the OCTA system, projection artifacts have been reported to artificially increase measurements of flow in the deep vascular network. However, a significant decrease in the flow measured in the deep plexus was demonstrated in eyes with BD in the present study. Moreover, upon visual inspection, we excluded images of the deep capillary plexus with significant artifacts. Fourth, vascular parameters were quantified only by 3-mm scans. However, a limited scanned area ensures quantification of the FAZ and VD from scans of the highest quality, given the trade-off between scan size and image resolution in the OCTA system. Furthermore, although the macular area scanned was small, we still detected a significant difference between the BD group and control group.

Using OCTA, this study demonstrated significantly reduced VD in deep capillary plexus in eyes with quiescent Behçet’s uveitis, especially in the inferior region, when compared with normal eyes. The retinal vasculature parameters in the OCTA images were associated with outer retinal damage. Our results suggest that OCTA with an algorithm has the potential to become a noninvasive, objective, and practical technique for evaluation of the retinal vasculature and for understanding the mechanisms underlying the various pathologic changes that occur during inflammatory attacks of Behçet’s uveitis.

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