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Special Issue

Retinal Capillary Network and Foveal Avascular Zone in Eyes with Vein Occlusion and Fellow Eyes Analyzed With Optical Coherence Tomography Angiography

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Purpose. To evaluate the perifoveolar retinal capillary network at different depths and to quantify the foveal avascular zone (FAZ) in eyes with retinal vein occlusion (RVO) compared with their fellow eyes and healthy controls using spectral-domain optical coherence tomography angiography (SD-OCTA).

Methods. We prospectively recruited 23 patients with RVO including 15 eyes with central RVO (CRVO) and 8 eyes with branch RVO (BRVO), their fellow eyes, and 8 age-matched healthy controls (8 eyes) for imaging on prototype OCTA software within RTVue-XR Avanti. The 3 × 3 mm and 6 × 6 mm en face angiograms of superficial and deep retinal capillary plexuses were segmented. Perifoveolar retinal capillary network was analyzed and FAZ was quantified.

Results. Decrease in vascular perfusion at the deep plexus was observed in all eyes with CRVO (8/8, 100%) and BRVO (6/6, 100%) without cystoid macular edema, and in 8 of 15 (53%) and 2 of 8 (25%) of the fellow eyes, respectively. Vascular tortuosity was observed in 13 of 15 (87%) CRVO and 5 of 8 (63%) BRVO eyes. Collaterals were seen in 10 of 15 (67%) CRVO and 5 of 8 (63%) BRVO eyes. Mean FAZ area was larger in eyes with RVO than their fellow eyes (1.13 ± 0.25 mm^2 versus 0.58 ± 0.28 mm^2; $P = 0.007$) and controls (1.13 ± 0.25 mm^2 versus 0.30 ± 0.09 mm^2; $P < 0.0001$), and in fellow eyes of RVO patients when compared to controls (0.58 ± 0.28 mm^2 versus 0.30 ± 0.09 mm^2; $P = 0.01$).

Conclusions. Spectral-domain OCTA reveals abnormalities at different levels of perifoveolar retinal capillary network and is able to quantify the FAZ in RVO. Longitudinal studies may be considered to evaluate the clinical utility of OCTA in RVO and other retinal vascular diseases.

Keywords: retinal vein occlusion, optical coherence tomography angiography, foveal avascular zone, perifoveal retinal capillary plexus, vascular perfusion

Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopathy.[1] The venous occlusion may result from external compression, internal vascular obstruction, or vascular wall pathology, and may occur either in the central retinal vein (central retinal vein occlusion, CRVO) or a branch of a retinal vein (branch retinal vein occlusion, BRVO). A leakage of fluid may occur from capillaries that are upstream from/proximal to the obstructed vein causing cystoid macular edema (CME) that can compromise central vision.[2] Prognosis and visual outcome is dependent on the amount of retinal ischemia and the presence or absence of CME.[3]

There is growing interest in visualization of blood flow in the retinal microvasculature at different capillary levels in retinal vascular disease, as recent reports suggest that specific diseases feature isolated deep capillary ischemia.[4,5] Fluorescein angiography (FA), traditionally used to evaluate retinal vascular circulation in RVO, is limited in that it is unable to individually visualize the different retinal capillary plexuses and therefore may fail to recognize deep capillary ischemia.[6,7] Importantly, the test involves intravenous dye injection and thus is not without risk, it cannot be repeated multiple times during a single patient visit, and is unpleasant to perform repeatedly due to its invasive nature.

In contrast, optical coherence tomography angiography (OCTA) is a new technique based on depth-resolved motion contrast imaging that operates by comparing the fluctuations in signal amplitude due to movement of erythrocytes within blood vessels relative to the static surrounding tissue. By enhancing these fluctuations in the optical coherence tomography (OCT) signal, a three-dimensional (3D) OCT angiogram is generated, allowing assessment of the retinal and choroidal microvasculature in a noninvasive fashion.[8,9] A unique property of OCTA in comparison to dye-based angiography is that it is a depth-resolved imaging technique, and thus is able to generate 3D angiograms that can be used to examine the retinal and choroidal capillary plexuses at varying depths.[10]

A recent report of a single case illustrated areas of absent blood flow on OCTA consistent with areas of ischemic capillary nonperfusion on FA in a patient with ischemic BRVO using a swept-source OCT prototype.[11] More recently, spectral-domain OCTA studies have shown reduced vascular perfusion primarily at the level of deep retinal vascular plexus in RVO.[12,13] The present study aims to evaluate the morphology of the perifoveolar retinal capillary network and quantify the area of foveal avascular zone (FAZ) in eyes with RVO compared with their fellow eyes and age-matched healthy eyes using the OCTA prototype software within a commercially available spectral-domain (SD)-OCT device.

Methods

Subjects

Thirty-three patients with RVO and eight age-matched healthy subjects were prospectively recruited from the Tufts Medical Center (New England Eye Center, Boston, MA, USA) and the Stein Eye Institute (University of California Los Angeles [UCLA], Los Angeles, CA, USA) from October 2014 through May 2015 for imaging using prototype OCTA software on a commercially available SD-OCT system. This study was approved by the institutional review boards of Tufts Medical Center and UCLA and adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act of 1996. Informed consent was obtained from all subjects in accordance with the Tufts Medical Center and UCLA institutional review boards prior to enrollment in the study.

The healthy subjects (control eyes) had a best-corrected visual acuity (BCVA) of 20/20 or better and underwent a dilated fundus examination to exclude any existing retinal or choroidal pathology. In addition, the fellow eyes of patients with RVO underwent a complete ophthalmic examination to exclude evidence of vascular obstruction or other concurrent retinal or choroidal pathology. Patients with RVO were diagnosed based on history, examination findings, and retinal imaging. They underwent complete ophthalmic examination including Snellen visual acuity; dilated fundus examination on slit-lamp biomicroscopy and indirect ophthalmoscopy; OCT imaging on commercially available SD-OCT (software version 6.0; Carl Zeiss Meditec Inc., Dublin, CA, USA); and FA (Heidelberg Spectralis, Spectralis HRA + OCT; Heidelberg Engineering, Inc., Heidelberg, Germany) in selected cases prior to OCTA imaging. The duration of diagnosis and details of prior treatment were obtained from review of the patients' charts. Patients were excluded if they had any previous or concomitant posterior segment pathologies that could confound the interpretation of OCTA such as severe nonproliferative or proliferative diabetic retinopathy and neovascular AMD. In addition, all subjects with a myopic refractive error greater than 6 diopters and those with suboptimum OCTA image quality due either to excessive blinking and/or motion artifact were also excluded.

OCTA Imaging

All subjects underwent imaging using prototype software within the SD-OCT device (RTVue XR Avanti with AngioVue OCTA software; Optovue, Inc., Fremont, CA, USA). This device operates at a wavelength of 840 nm, a bandwidth of 45 nm, and a speed of 70,000 A-scans per second. We used the 3×3 mm and 6×6 mm volumes centered at the fovea, each consisting of 304 A-scans per B-scan for a total of 304 B-scans involving two repeated B-scans at a given retinal location that takes ~2.6 seconds to acquire. Trained operators performed all OCTA imaging. The split spectrum amplitude decorrelation angiography algorithm[14] generates 3D, 3×3 mm and 6×6 mm en face OCTA images for each eye using the automated software segmentation that involves orthogonal registration and merging of two consecutive volumes to improve signal. Three-dimensional OCTA images were coregistered with the cross-sectional OCT images (B-scans) obtained concurrently allowing assessment of both the microvasculature of the retina and its structure in tandem.

Segmentation and Image Analysis

The OCTA software (Optovue, Inc.) allows manual alteration of its automated segmentation lines on the coregistered OCT B-scans, and thus the relative depth and thickness of the imaged plane can be changed. This function was used to manually divide the perifoveolar retinal capillary network into the superficial and deep plexus for analysis (Fig. 1). The superficial retinal capillary plexus slab was taken from the inner limiting membrane (ILM) or the vitreoretinal interface and the inner plexiform layer to incorporate the ganglion cell layer (GCL); and the deep retinal capillary plexus slab was taken from the inner plexiform layer and the outer plexiform layer (OPL) to incorporate the inner nuclear layer (INL). In cases where significant retinal thinning or presence of CME caused distortion of the retinal architecture, manual delineation of the superficial and deep retinal capillary plexuses was not feasible and only the unsegmented entire retinal angiogram from the ILM to the RPE was used for analysis.

Analysis of all OCTA images and coregistered OCT B-scans of both eyes in all subjects was performed at the New England Eye Center by two independent trained readers of the Boston Image Reading Center (MA, MABF). The readers assessed the presence or absence of the following morphologic features of the perifoveolar retinal capillary network:

1. Decrease in vascular perfusion: defined as dark or gray areas with a hypointense hue on OCTA and with irregular borders created by the well-perfused adjacent capillaries;
2. Vascular tortuosity: defined as any curving, angulation, kinking, and/or spiral twisting of vessels; and
3. Presence of collaterals: defined as either vessel to vessel channels appearing as a bunch of tortuous vessels around the blockage site or a single long channel traversing a blocked retinal segment.

Presence or absence of CME was also noted. Eyes with CME were not used for assessment of a decrease in vascular perfusion, as CME may cause signal attenuation caused by a shadowing artifact and potential overestimation of a decrease in vascular perfusion (described below). Both 3×3 mm and 6×6 mm OCTA images were analyzed. Vascular perfusion was assessed at the superficial plexus and deep plexus levels. For assessment of vascular tortuosity and collateral formation, only

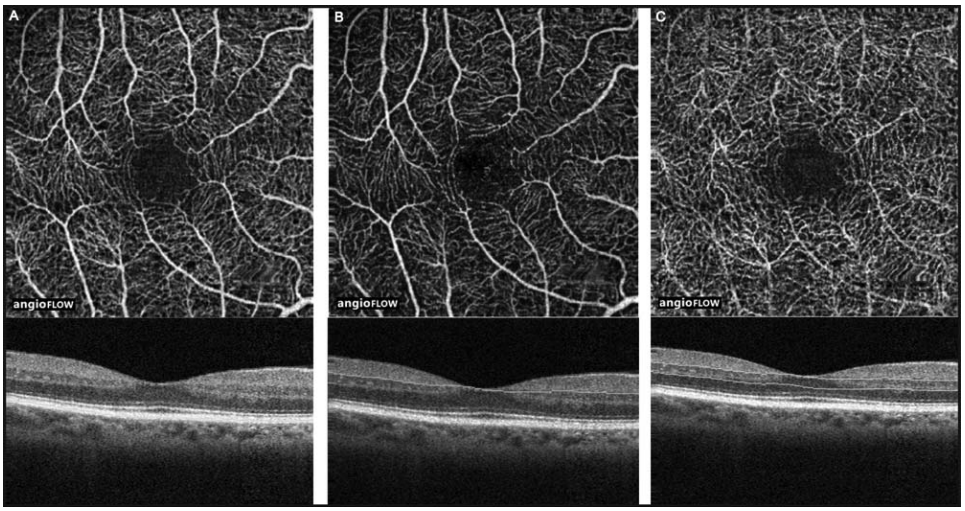

FIGURE 1. Manual segmentation of the retina in a healthy eye using the OCTA software to generate en face retinal angiograms at different depths for analysis. (**A**) Unsegmented 3 × 3 mm OCTA and the corresponding coregistered B-scan with the entire retinal angiogram from the ILM to the RPE. (**B**) En face 3 × 3 mm OCTA and the corresponding coregistered B-scan showing segmentation to obtain the superficial retinal capillary plexus from the ILM to the outer aspect of the GCL. (**C**) En face 3 × 3 mm en face OCTA and the corresponding coregistered B-scan showing segmentation to obtain the deep retinal capillary plexus from the inner aspect of the IPL to the outer aspect of the OPL.

the unsegmented OCTA images were analyzed. Any disagreements between the two observers were resolved by open adjudication to reach a final decision on the presence or absence of a particular feature.

Additionally, the 3 × 3 mm unsegmented OCTA images were used to quantify the area of FAZ by delineating the innermost vessels surrounding the area of absent vessels at the fovea using the free-hand tool on ImageJ (http://imagej.nih.gov/ij/; provided in the public domain by the National Institute of Health, Bethesda, MD, USA). The pixel dimensions of the original images were noted before importing them into ImageJ. The measurements were rescaled to the squared millimeter unit on the basis of the original pixel dimensions of the images. Two observers (MA, MABF) independently performed the measurements and an average of the measurements of the two observers were used for the purposes of analysis.

Statistical Analysis

All data was expressed as mean ± SD. Unpaired Student's *t*-tests were used to determine the difference in the ages of RVO patients and healthy subjects and to determine the difference in the FAZ measurements between eyes with RVO and healthy eyes. Paired Student's *t*-tests were used to determine the difference in FAZ measurements between eyes with RVO and their fellow eyes. An intraclass correlation coefficient was employed to determine the interobserver agreement for FAZ measurements. A 95% confidence interval (CI) and a 5% level of significance were adopted; therefore, results with a *P* value less than or equal to 0.05 were considered significant. All statistics were performed using graphing (Graph Pad Prism 5.0 software for Macintosh; GraphPad Software, Inc., La Jolla, CA, USA) and statistics software (SPSS, version 19; IBM Corp., Chicago, IL, USA).

TABLE 1. Demographics and Clinical Characteristics of Patients With RVO

RVO eyes, *n*	23 (23)
Mean Age ± SD	66.71 ± 12.38
Sex, males/female	11/12
Race, Caucasian/Asian/Hispanic	18/3/2
Range of BCVA	20/20–CF at 7′
Mean duration of RVO, mo (mean, range)	5 (1–48)
CRVO eyes, *n*	15
BRVO eyes, *n*	8

CF, counting fingers.

RESULTS

Thirty-three patients with unilateral RVO were imaged using OCTA. We excluded four patients due to concomitant unrelated retinal disease and six patients were excluded due to suboptimal image quality, leaving 23 eyes of 23 patients with unilateral RVO for the purpose of analysis. Of these, 15 patients had CRVO and 8 patients had BRVO. A total of 10 of 15 (67%) patients with CRVO and 5 of 8 (63%) patients with BRVO had previously been treated with anti-VEGF injections. Fluorescein angiography (FA) was performed in 10 of these patients within 12 weeks of the OCTA. No interventions were performed between the OCTA and FA imaging dates in these patients. All 23 fellow eyes of these patients and 8 eyes of 8 age-matched healthy subjects were also imaged using OCTA and analyzed.

The mean age of RVO patients was 66.71 ± 12.38 years and that of the healthy subjects was 64.80 ± 8.3 years. There was no significant difference in the mean ages of the RVO patients and the healthy subjects ($P = 0.68$). The demographics and clinical characteristics of RVO patients are presented in Table 1. Analysis of OCTA of healthy eyes demonstrated a clearly demarcated FAZ bounded by a distinct foveal vascular ring with a relatively lower capillary density than in the parafoveal regions. The superficial retinal capillary plexus in healthy eyes appeared more similar to the unsegmented OCTA image than did the deep retinal capillary plexus, and consisted of capillary segments that mostly traveled concentrically around the fovea, while the deep retinal capillary plexus consisted of a more homogeneous lattice-like or web-like pattern of vessels with some small discontinuous segments (Fig. 1). This pattern was consistent with that described previously.[9]

Cystoid macular edema was noted on coregistered OCT B-scans in 7 of 15 (47%) and 2 of 8 (25%) eyes with CRVO and BRVO, respectively. In the remaining 8 of 15 (53%) eyes with CRVO and 6 of 8 (75%) eyes with BRVO without CME, a

Table 2. Percent Visualization of the Morphologic Features and Area of FAZ in Eyes With CRVO and BRVO, Fellow Eyes, and Healthy Eyes Using OCTA

	CRVO		BRVO		
	Affected Eyes	**Fellow Eyes**	**Affected Eyes**	**Fellow Eyes**	**Healthy Eyes**
CME, *n* (%)	7/15 (47%)	0/15 (0%)	2/8 (25%)	0/8 (0%)	0/8 (0%)
Percent visualization of features of retinal microvasculature					
Decreased vascular perfusion (assessed at the deep plexus)*	8/8 (100%)	8/15 (53%)	6/6 (100%)	2/8 (25%)	0/8 (0%)
Vessel tortuosity	13/15 (87%)	9/15 (60%)	5/8 (63%)	2/8 (25%)	0/8 (0%)
Collaterals	10/15 (67%)	0/15 (0%)	5/8 (63%)	0/8 (0%)	0/8 (0%)
FAZ area in mm^2 (mean ± SD)	1.26 ± 0.3	0.66 ± 0.22	0.88 ± 0.33	0.45 ± 0.18	0.30 ± 0.09

* Decreased vascular perfusion was assessed only in eyes without cystoid macular edema.

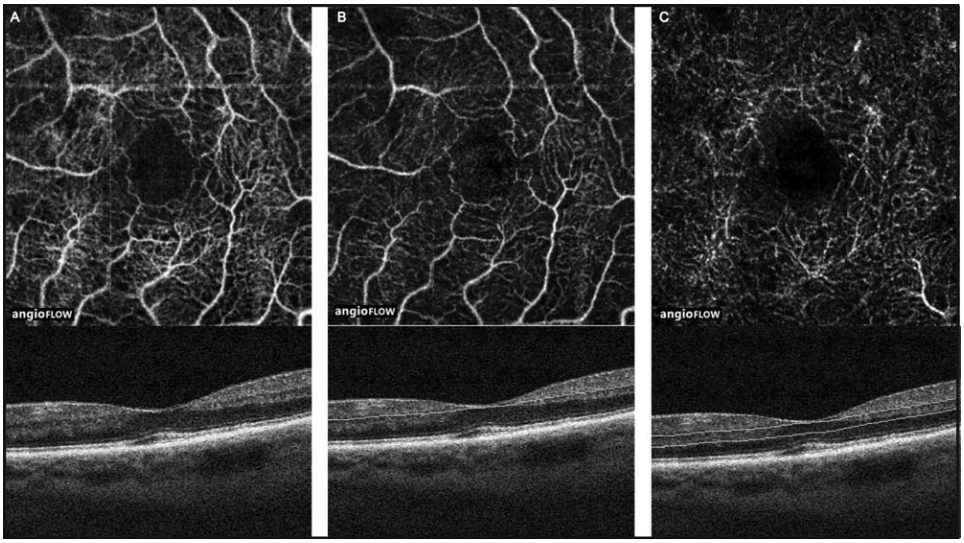

Figure 2. Segmentation of the retina using the OCTA software at different depths in an eye with CRVO without CME showing decreased vascular perfusion more pronounced in the deep retinal vascular plexus. (**A**) Unsegmented 3 × 3 mm OCTA and the corresponding coregistered B-scan with the entire retinal angiogram from the ILM to the RPE. (**B**) En face 3 × 3 mm OCTA and the corresponding coregistered B-scan showing segmentation to obtain the superficial retinal capillary plexus from the ILM to the outer aspect of the GCL. (**C**) En face 3 × 3 mm OCTA and the corresponding coregistered B-scan showing segmentation to obtain the deep retinal capillary plexus from the inner aspect of the IPL to the outer aspect of the OPL. Note the decrease in vascular perfusion in this eye that is more pronounced in the deep retinal capillary plexus.

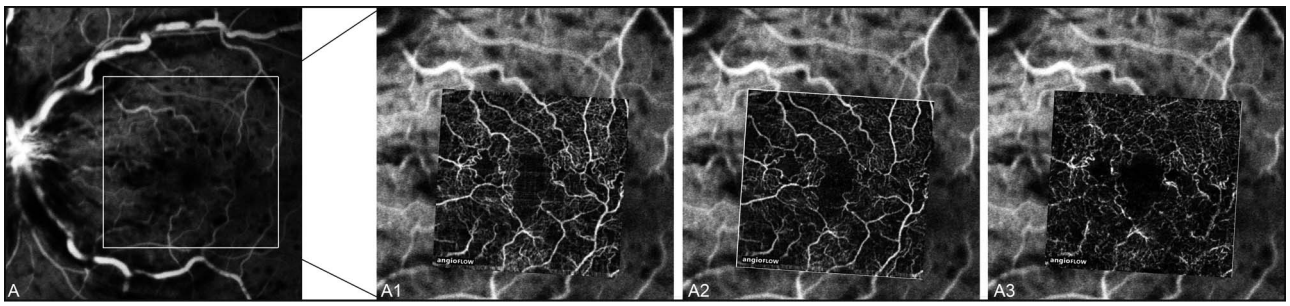

Figure 3. Fluorescein angiography (early frame) with superimposed OCTA at different depths showing decreased vascular perfusion in an eye with central retinal vein occlusion without cystoid macular edema. (**A**) Fluorescein angiography (early frame) showing decreased vascular perfusion. (**A1**) Unsegmented 3 × 3 mm OCTA superimposed on an early frame FA showing decreased vascular perfusion consistent with FA. (**A2**) En face 3 × 3 mm OCTA segmented to obtain the superficial retinal capillary plexus superimposed on an early frame FA showing decreased vascular perfusion consistent with FA. (**A3**) En face 3 × 3 mm OCTA segmented to obtain the deep retinal capillary plexus superimposed on an early frame FA showing decreased vascular perfusion consistent with FA. Note that the retinal capillary plexus is better visualized on OCTA than FA and that the deep retinal capillary plexus shows a more pronounced decrease in vascular perfusion.

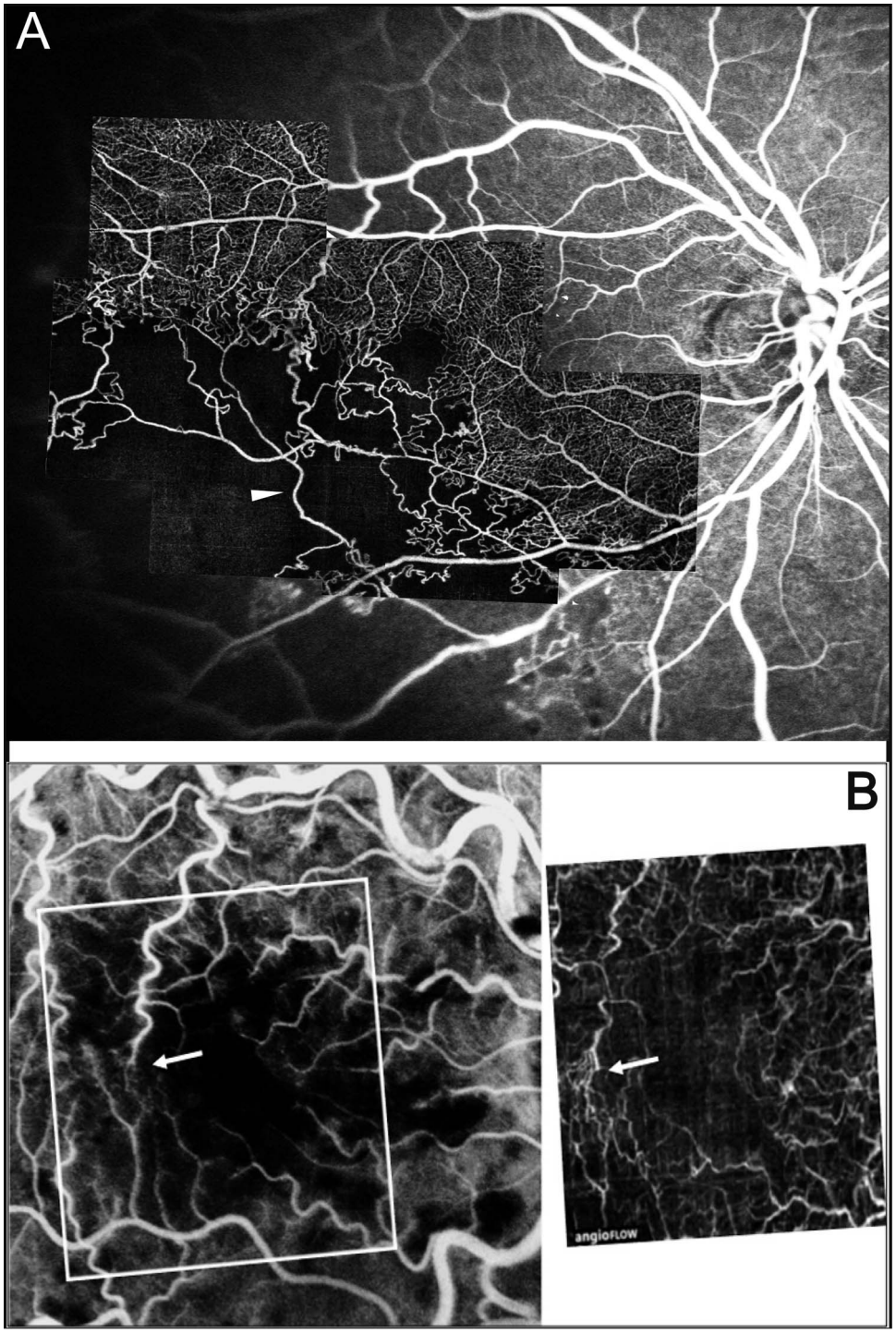

FIGURE 4. Optical coherence tomography angiography correlated with FA showing structural vascular changes in eyes with RVO. (**A**) Unsegmented 3 × 3 mm OCTA acquired in multiple regions, montaged and then superimposed on an early frame FA in an eye with BRVO. Note the vascular tortuosity, decrease in vascular perfusion and presence of collateral vessels (*white arrow*) observed on the OCTA. (**B**) Unsegmented 3 × 3 mm OCTA and early frame FA side by side from an eye with CRVO. Note the decrease in vascular perfusion on OCTA that correlates with the capillary nonperfusion on FA. Also note the vascular tortuosity and presence of collaterals (*white arrow*).

decrease in vascular perfusion at the deep retinal vascular plexus was observed in all eyes with CRVO (8/8, 100%) and BRVO (6/6, 100%). At the superficial retinal capillary plexus and with the unsegmented OCTA images, the decrease in vascular perfusion was observed less frequently and was noted in five of eight (63%) of eyes with CRVO and three of six (66%) eyes with BRVO without CME. In the fellow eyes, 8 of 15 (53%) and 2 of 8 (25%) fellow eyes of patients with CRVO and BRVO, respectively, demonstrated a decrease in vascular perfusion at the level of deep retinal capillary plexus, while only 3 of 15 (20%) and 0 of 8 (0%) fellow eyes of patients with CRVO and BRVO, respectively, showed this feature at the level of the superficial plexus and with the unsegmented OCTA images. The decrease in vascular

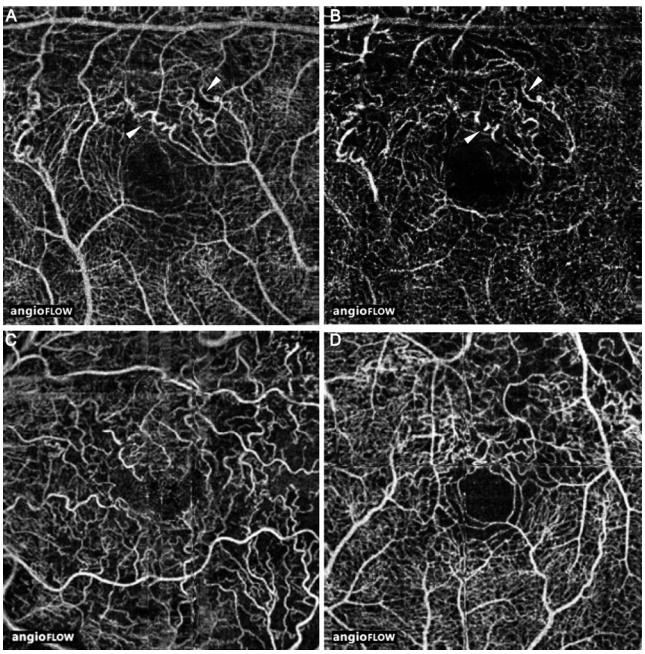

FIGURE 5. Optical coherence tomography angiography showing structural vascular changes in eyes with RVO. (**A**) Unsegmented 3 × 3 mm OCTA showing collateral formation (*white arrows*) in an eye with BRVO. (**B**) En face 3 × 3 mm OCTA showing segmentation to obtain the deep retinal capillary plexus of the same eye as in (**A**) showing the collaterals traversing into the retina down to the level of the deep retinal capillary plexus (*white arrows*). (**C**) Unsegmented 3 × 3 mm OCTA showing vascular tortuosity in an eye with CRVO. (**D**) Unsegmented 3 × 3 mm OCTA in the fellow eye of a patient with CRVO showing some vascular tortuosity in the *upper right region* of OCTA image.

perfusion was consistent with the capillary nonperfusion seen on FA (Table 2; Figs. 2–4).

Vascular tortuosity was observed in 13 of 15 (87%) and 5 of 8 (63%) of eyes with CRVO and BRVO, respectively (Table 2; Figs. 4, 5). This feature was also seen in some of the fellow eyes of patients with RVO (Table 2). Collaterals were observed in 10 of 15 (67%) and 5 of 8 (63%) of eyes with CRVO and BRVO, respectively (Figs. 4, 5), but none were observed in any fellow eyes. Interestingly, collaterals could be seen traversing into the retina down to the level of the deep retinal capillary plexus in some cases (Fig. 5). Neither a decrease in vascular perfusion, nor vascular tortuosity or collaterals was observed in the healthy control eyes (Table 2).

Figure 6 demonstrates an eye with CME secondary to BRVO with improvement following treatment with intravitreal anti-VEGF therapy on follow-up OCTA imaging. It also demonstrates that the presence of CME makes delineation of the retinal layers for manual segmentation of the superficial and deep retinal capillary plexuses challenging and causes a shadowing effect that may potentially overestimate a decrease in vascular perfusion.

The mean area of FAZ measured on the 3 × 3 mm unsegmented OCTA images was larger in eyes with RVO when compared with their fellow eyes (1.13 ± 0.25 mm^2 versus 0.58 ± 0.28 mm^2; $P = 0.007$) and to healthy eyes (1.13 ± 0.25 mm^2 versus 0.30 ± 0.09 mm^2; $P < 0.0001$). In addition, the mean FAZ area in the fellow eyes of RVO patients was larger than that of the healthy eyes (0.58 ± 0.28 mm^2 versus 0.30 ± 0.09 mm^2; $P = 0.01$). There was no significant difference in the mean FAZ area between males and females, respectively, in eyes with RVO (1.09 ± 0.23 mm^2 versus 1.15 ± 0.27 mm^2; $P = 0.5$), fellow eyes of RVO patients (0.55 ± 0.27 mm^2 versus 0.60 ± 0.29 mm^2; $P = 0.6$) and healthy eyes (0.31 ± 0.08 mm^2 versus 0.28 ± 0.12 mm^2; $P = 0.7$).

On a subgroup analysis, the mean area of FAZ was larger in eyes in CRVO when compared with their respective fellow eyes (1.26 ± 0.38 mm^2 versus 0.66 ± 0.22 mm^2; $P < 0.0001$) and healthy eyes (1.26 ± 0.38 mm^2 versus 0.30 ± 0.09 mm^2; $P < 0.0001$) and in eyes with BRVO compared with their respective fellow eyes (0.88 ± 0.33 mm^2 versus 0.45 ± 0.18 mm^2; $P = 0.006$) and healthy eyes (0.88 ± 0.33 mm^2 versus 0.30 ± 0.09 mm^2; $P = 0.0003$; Table 2). While the fellow eyes of patients with CRVO had a larger mean FAZ than that of healthy eyes (0.66 ± 0.22 mm^2 versus 0.30 ± 0.09 mm^2; $P = 0.0003$), there was no significant difference in the FAZ area in the fellow eyes of patients with BRVO when compared with healthy eyes (0.45 ± 0.18 mm^2 versus 0.30 ± 0.09 mm^2; $P = 0.08$). The intraclass correlation coefficient for FAZ measurements between the two observers was 0.996 (95% CI, 0.993–0.998).

DISCUSSION

In this study, the authors describe the morphology of the perifoveolar retinal capillary network at different levels and quantify the FAZ in eyes with RVO using prototype OCTA software on a commercially available SD-OCT device and compare these findings to their fellow eyes and age-matched healthy eyes. In patients without CME, there was a decrease in vascular perfusion that occurred more frequently and was also more pronounced in the deep retinal vascular plexus than in the superficial retinal plexus. Other findings included vessel tortuosity and collateral formation, and an increase in the FAZ size in eyes with RVO. To the authors' knowledge, this is the first study that quantifies the FAZ in eyes affected with retinal vein occlusion and their fellow eyes.

The retinal capillary network is arranged in morphologically distinct layers. The superficial retinal capillary plexus is located predominantly within the ganglion cell layer, the deep retinal capillary plexus is located within the INL, and perpendicularly positioned vessels interconnect these plexuses.[15] Although an intermediate capillary plexus is also present,[15] the current OCTA technology does not allow separation of the intermediate plexus from deep retinal capillary plexus. Hence, the intermediate plexus is assessed as a complex with the deep retinal capillary plexus using OCTA. Evidence suggests that the superficial and the deep retinal capillary plexuses may be disproportionately affected in retinal vascular disease.[4,5,16–18] While retinal vascular changes in RVO have been previously described using conventional imaging techniques such as FA, precise evaluation of the vasculature at different capillary levels is not feasible using FA.[8] Optical coherence tomography angiography offers a depth-resolved method of analyzing the retinal vasculature allowing differentiation of the superficial and deep retinal capillary plexuses. The present study shows diminished vascular perfusion in eyes with RVO that occurs more frequently and also appears to be more pronounced in the deep retinal capillary plexus. These findings are consistent with the recent studies using OCTA in eyes with vein occlusions.[12,13] Since the superficial retinal capillary plexus is directly connected to the retinal arterioles, it may have greater perfusion pressure. The deep retinal capillary plexus is primarily formed of venous collecting channels as shown in an animal model of RVO using dye-based angiography and later in normal subjects using OCTA and thus, may inherently be more susceptible to high venous pressures.[16,17] In addition, the deep retinal capillary plexus is located in a watershed-like area where the oxygen saturation may be lower than the inner and

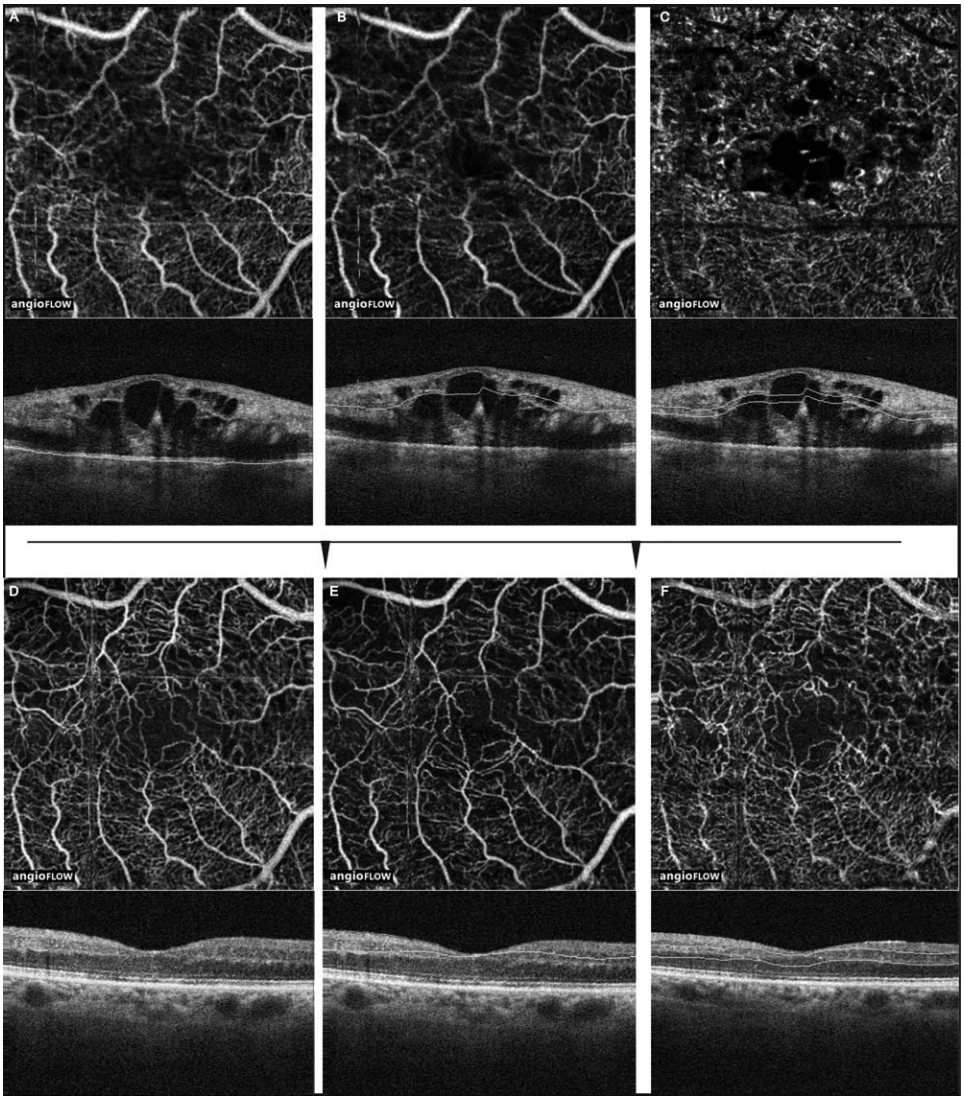

FIGURE 6. Segmentation of the retina at different depths in an eye with BRVO with CME with improvement following treatment on follow-up imaging and illustration of the effect of CME on segmentation and interpretation of OCTA. (**A**) Unsegmented 3 × 3 mm OCTA and the corresponding coregistered B-scan before treatment (**B**) En face 3 × 3 mm OCTA segmented to obtain the superficial retinal capillary plexus and the corresponding coregistered B-scan before treatment. (**C**) En face 3 × 3 mm OCTA segmented to obtain the deep retinal capillary plexus and the corresponding coregistered B-scan before treatment. Note the presence of CME in the B-scans in (**A–C**) that makes delineation of the retinal layers for manual segmentation of the superficial and deep retinal capillary plexuses difficult in addition to its shadowing effect on the corresponding OCTA images that may potentially overestimate a decrease in vascular perfusion. (**D**) Unsegmented 3 × 3 mm OCTA and the corresponding coregistered B-scan after 2 months following treatment with 2 injections of intravitreal bevacizumab. (**E**) En face 3 × 3 mm OCTA segmented to obtain the superficial retinal capillary plexus and the corresponding coregistered B-scan after 2 months following treatment with 2 injections of intravitreal bevacizumab. (**F**) En face 3 × 3 mm OCTA segmented to obtain the deep retinal capillary plexus and the corresponding coregistered B-scan after 2 months following treatment with 2 injections of intravitreal bevacizumab. Note that the CME has resolved in the B-scans in (**D–F**) making it easier to manually segment the retina to obtain the OCTA images at different depths of the retinal capillary network. Even so, areas of decreased vascular perfusion are visible especially in the deep retinal vascular plexus (**F**).

outer retina.[18] This anatomic and functional distribution of the retinal vasculature may partly explain why the deep retinal capillary plexus is more vulnerable to ischemic insults and more markedly affected in eyes with RVO. The depth and area of decreased vascular perfusion, in addition to the presence or absence of CME, may play a role in the visual prognosis in these eyes; however, future studies would be necessary to make any definite conclusions.

Evidence suggest that fellow eyes of patients with RVO may be at an increased risk of vein occlusion compared to the general population.[3] This may be associated with general risk factors such as increasing age, hypertension, diabetes and arteriosclerosis. Even before an occlusive event, these fellow eyes may be structurally compromised.[19] A recent study showed decreased microvascular density in the fellow eyes of patients with CRVO using adaptive optics scanning light ophthalmoscope FA.[20] The present study found a decrease in vascular perfusion that was more pronounced in the deep retinal vascular plexus and vessel tortuosity using OCTA in some fellow eyes of patients with RVO. Given the high incidence of bilateral occurrence in this disease, it is possible that occult RVO may have been present in these eyes and is

only detectable with OCTA analysis. These preliminary findings suggest that OCTA may be useful in elucidating prior occlusive disease or possibly early pathologic changes that may signal a future occlusive event. However, future longitudinal studies are needed to make any final deductions.

The foveal capillary network forms a ring at the margin of the fovea producing a capillary-free region called the FAZ.[14,21,22] The size of the FAZ has been reported to be larger in diseases such as diabetic retinopathy using FA.[23] However, accurate FAZ measurements are usually limited by the quality of FA images.[24,25] In the present study, FAZ was easily delineated using OCTA and was significantly larger in eyes with RVO compared with their fellow eyes and to healthy eyes. In addition, it was also larger in the fellow eyes of patients with RVO compared with healthy eyes. An enlargement of FAZ in eyes with RVO may represent macular ischemia and could be an important feature contributing to visual acuity impairment in these eyes.[26]

There are some obvious advantages of OCTA in comparison to FA, including its rapid, easy, and comfortable image acquisition, depth resolution, lack of a need of invasive dye injection, depth-resolved evaluation of the retinal capillary microvasculature, and more precise FAZ quantification. Limitations of OCTA include a small field of view, motion artifacts in patients with poor fixation or poor vision, and absence of dynamic flow and vascular leakage information. To enhance field of view, multiple images may be stitched into a montage image. Eye tracking hardware and software can control for motion artifacts. However, an absence of dynamic blood flow and vascular leakage information that conventional FA is able to provide are still the main limitations of OCTA in comparison to FA. The present study does not attempt to make a direct comparison of OCTA with FA in eyes with RVO since the patients in this cohort did not usually receive an FA on the same visit as the OCTA imaging.

Notably, assessment of vascular nonperfusion on OCTA may be limited in the presence of CME in eyes with RVO owing to signal attenuation caused by a shadowing artifact of the fluid accumulation (Fig. 6). The present study analyzed a decrease in vascular perfusion only in RVO eyes without CME to ascertain that the appearance of this feature was a true depiction instead of the result of an imaging artifact. In addition, assessment of vascular nonperfusion on OCTA may also be limited due to a shadowing artifact in the presence of intraretinal hemorrhage as well as from projection artifacts of the superficial vessels on to the deep retinal capillary plexus. Recent studies have employed advanced image processing and volume-rendering analysis of the deep retinal capillary plexus to obviate these limitations. Integration of these methods into commercial OCTA instruments is expected to improve the utility and diagnostic accuracy of OCTA.[27,28] Further, foveal microstructure may be altered following anti-VEGF treatment in retinal vascular disease.[29] Future studies that compare treatment-naïve and previously treated eyes with RVO will help determine the effect of anti-VEGF treatment on the observed changes in the retinal microvasculature in this disease.

In conclusion, the present study assessed the morphology of the perifoveolar retinal capillary network at different levels and quantified the FAZ in eyes with RVO and compared these findings to the fellow eyes and healthy controls using OCTA. It demonstrated a predilection for ischemia of the deep retinal capillary plexus to occur in both affected and fellow eyes of patients with RVO and also demonstrated enlarged FAZ areas in these eyes. Future longitudinal studies involving a large number of patients are expected to help understand the natural progression and pathophysiology of RVO and further delineate the clinical utility of OCTA in RVO and other retinal vascular diseases.

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