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Swept-source OCT angiography imaging of the foveal avascular zone and macular capillary network density in diabetic retinopathy

Al-Sheikh, Mayss; Akil, Handan; Pfau, Maximilian; Sadda, SriniVas R

Abstract: PURPOSE We compared the area of the foveal avascular zone (FAZ) and macular capillary network density at different retinal layers using swept-source optical coherence tomography angiography (OCT-A) in normal individuals and patients with diabetic retinopathy (DR). METHODS Images (a 3 × 3 mm cube centered on the fovea) were acquired in 40 eyes of 22 normal individuals and 28 eyes of 18 patients with varying levels of DR using a swept-source OCT-A device (central wavelength 1050 nm; A-scan-rate of 100,000 scans per second). En face images of the retinal vasculature were generated from the superficial and deep retinal layers (SRL/DRL). Quantitative analysis of the vessel density (VD) and FAZ area was performed. Vessel density was assessed as the ratio of the retinal area occupied by vessels. RESULTS Among the DR subjects (mean age, 72 years; 61% male), 35.7% of the eyes had mild, 35.7% moderate, and 7.1% severe nonproliferative DR (NPDR), and 21.4% and proliferative DR (PDR). The mean FAZ area in patients with DR and in normal individuals was 0.518 and 0.339 mm2, respectively, for the SRL ($P = 0.003$), and 0.615 and 0.358 mm2, respectively, for the DRL ($P < 0.001$). The mean VD (ratio) at the SRL and DRL was statistically significantly lower in patients with DR (SRL, $P < 0.001$; DRL, $P = 0.028$). CONCLUSIONS Swept-source OCT-A of the microcirculation in eyes of patients with DR can be used to quantitatively demonstrate alterations in the FAZ and VD in the SRL/DRL of the macula compared to normal eyes. Future longitudinal studies may use these metrics to evaluate changes over time or in response to treatment.

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Swept-Source OCT Angiography Imaging of the Foveal Avascular Zone and Macular Capillary Network Density in Diabetic Retinopathy

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Purpose. We compared the area of the foveal avascular zone (FAZ) and macular capillary network density at different retinal layers using swept-source optical coherence tomography angiography (OCT-A) in normal individuals and patients with diabetic retinopathy (DR).

Methods. Images (a 3 × 3 mm cube centered on the fovea) were acquired in 40 eyes of 22 normal individuals and 28 eyes of 18 patients with varying levels of DR using a swept-source OCT-A device (central wavelength 1050 nm; A-scan-rate of 100,000 scans per second). En face images of the retinal vasculature were generated from the superficial and deep retinal layers (SRL/DRL). Quantitative analysis of the vessel density (VD) and FAZ area was performed. Vessel density was assessed as the ratio of the retinal area occupied by vessels.

Results. Among the DR subjects (mean age, 72 years; 61% male), 35.7% of the eyes had mild, 35.7% moderate, and 7.1% severe nonproliferative DR (NPDR), and 21.4% and proliferative DR (PDR). The mean FAZ area in patients with DR and in normal individuals was 0.518 and 0.339 mm^2, respectively, for the SRL ($P = 0.003$), and 0.615 and 0.358 mm^2, respectively, for the DRL ($P < 0.001$). The mean VD (ratio) at the SRL and DRL was statistically significantly lower in patients with DR (SRL, $P < 0.001$; DRL, $P = 0.028$).

Conclusions. Swept-source OCT-A of the microcirculation in eyes of patients with DR can be used to quantitatively demonstrate alterations in the FAZ and VD in the SRL/DRL of the macula compared to normal eyes. Future longitudinal studies may use these metrics to evaluate changes over time or in response to treatment.

Keywords: optical coherence tomography, angiography, diabetic retinopathy, vessel density, foveal avascular zone

Diabetic retinopathy (DR) is the leading cause of visual impairment and blindness in most developed countries.[1,2] Since the number of patients with DR is expected to grow, and early detection and intervention is useful in terms of preventing severe vision loss,[3–6] further investigations are needed regarding new methods for evaluating the microvascular status and therapeutic effect of treatment.

The macular microvasculature is a complex system consisting of three capillary plexuses, which are responsible for the blood supply of the inner retina: the superficial retinal layer (SRL) located in the retinal nerve fiber layer, and the two plexuses located at the inner and outer border of the inner nuclear layer, which together make up the deep retinal layer (DRL).[7] The outer retina and foveal avascular zone depend on diffusion from the choroidal circulation.[8] It has been reported that retinal blood flow decreases in patients with type 2 diabetes mellitus who have no or mild DR, suggesting that the retinal microvasculature becomes impaired in early-stage DR, even in patients with no evidence of retinopathy.[9] Previous histologic studies have shown capillary nonperfusion to be an important feature of this vascular disease.[10] In vivo, the gold standard to screen for DR is dilated biomicroscopic fundus examination. Fluorescein angiography (FA) is more sensitive than examination to detect early microvascular changes.[11] Previous studies have shown enlargement of the intercapillary areas in patients with DR and decreased capillary perfusion density.[12] Since FA takes several minutes and requires the administration of an intravenous dye, the technique is not optimal for screening or frequent longitudinal assessments.[11,13] In addition, leakage of fluorescein dye and the superimposition of capillaries from different retinal layers onto a single two-dimensional FA image have hindered a more detailed investigation of the microvasculature by FA.

This is particularly important, since recent evidence suggests that a diabetic retinal neuropathy and loss of photoreceptors may precede the development of an overt retinal vasculopathy.[14] The deep retinal capillary circulation, however, supplies nourishment to the photoreceptor zones (Henle's layer), especially during systemic hypoxia as the choroidal vasculature fails to autoregulate the blood supply.[15] One wonders whether subtle, selective alterations to the deep capillary plexus, not visible by FA, could have a role in the pathophysiology of early diabetic retinal neuropathy.

Optical coherence tomography angiography (OCT-A) is a novel, noninvasive method of visualizing the retinal microcirculation in a depth-resolved fashion, allowing the superficial

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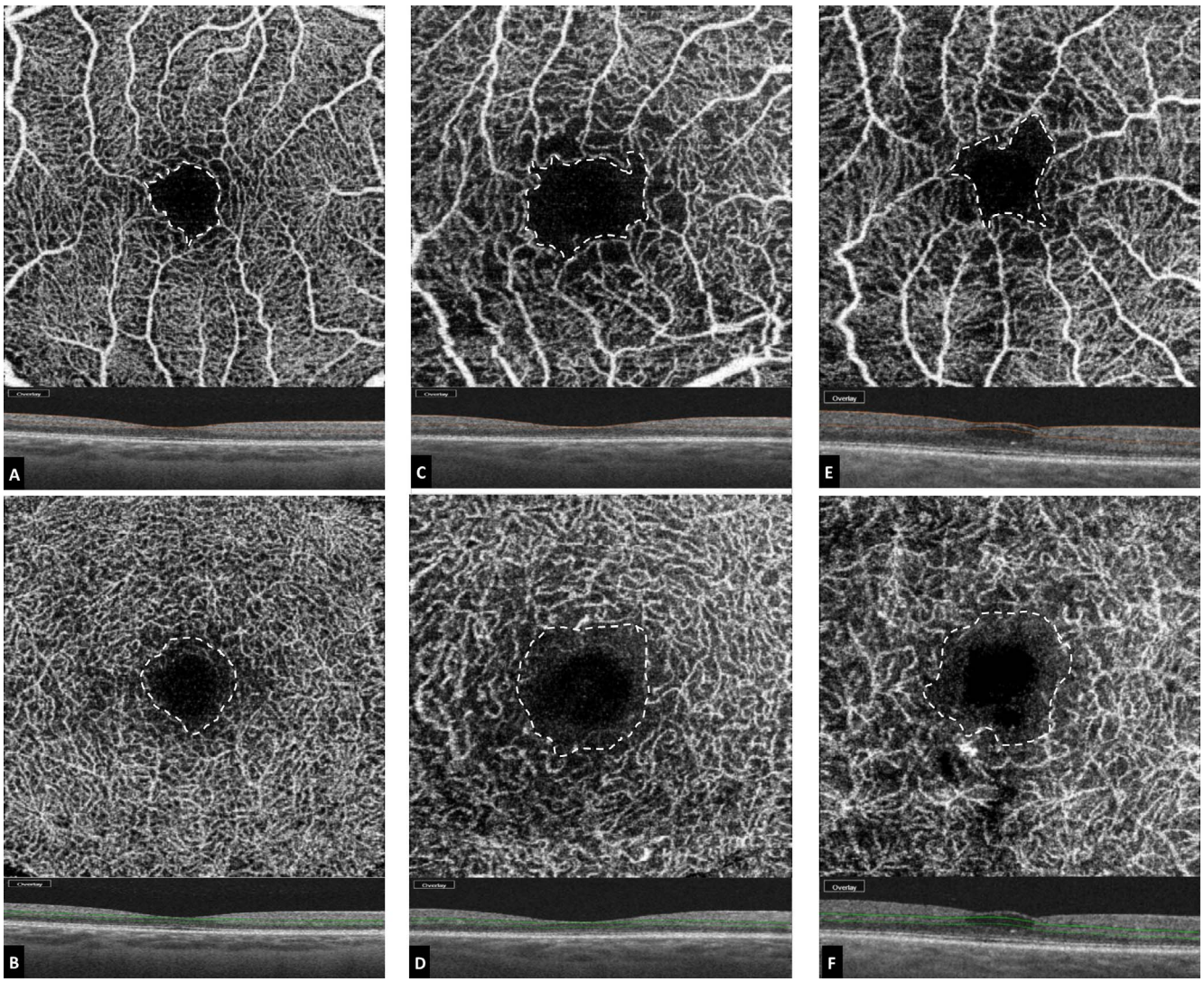

FIGURE 1. Swept-source OCT-A images of three subjects centered on the fovea. (**A**, **B**) En face projection image of the foveal avascular zone (*outlined*) of the superficial and DRLs in a healthy individual with segmentation. (**C**, **D**) Corresponding en face projection images of a patient with DR without DME. (**E**, **F**) En face OCT-A images of a patient with DR with DME.

and deep capillary plexuses to be studied separately.[16,17] In addition, because contrast between retinal vessels and surrounding tissues is high, OCT-A lends itself to segmentation and quantification of the retinal microvasculature. We and others have shown that the superficial and deep capillary circulation can be quantified reliably, with important quantitative and qualitative differences apparent between the deep and superficial layers.[18–20] Ishibazawa et al.[21] described, in a pilot study, the pathologic vascular changes of DR, including microaneurysms, retinal nonperfusion, and neovascularization. de Carlo et al.[22] found enlargement and remodeling of the foveal avascular zone (FAZ) area in eyes of subjects with diabetes but without clinical DR. Quantitative comparisons between normal eyes and eyes with DR, however, still are limited, particularly for swept-source based OCT-A devices. Swept-source OCT technology uses longer-wavelength infrared light with less sensitivity roll-off with depth compared to conventional spectral-domain OCT. This allows a deeper penetration into tissue and better imaging through optical opacities. While this sensitivity benefit may be most apparent for choroidal vascular imaging, it also may be relevant to situations with marked retinal thickening, such as with severe macular edema. In addition, different OCT-A instruments use different proprietary flow detection and segmentation algorithms, which may yield different results. Thus, the microvascular abnormalities must be studied with various devices to better define the potential discrepancies.

The aim of this study was to evaluate the FAZ area and perifoveal capillary network density in the SRL and DRL in patients with varying severities of DR, and to compare the findings to those of normal individuals using a swept-source OCT-A.

METHODS

Study Population

This study was approved by the Institutional Review Board of the University of California Los Angeles (UCLA) and conducted in accordance with the ethical standards stated in the Declaration of Helsinki. Informed consent was obtained from all examined patients and healthy individuals to participate in this research. The study included 28 eyes of 18 diabetic patients with DR and 40 eyes of 22 healthy individuals.

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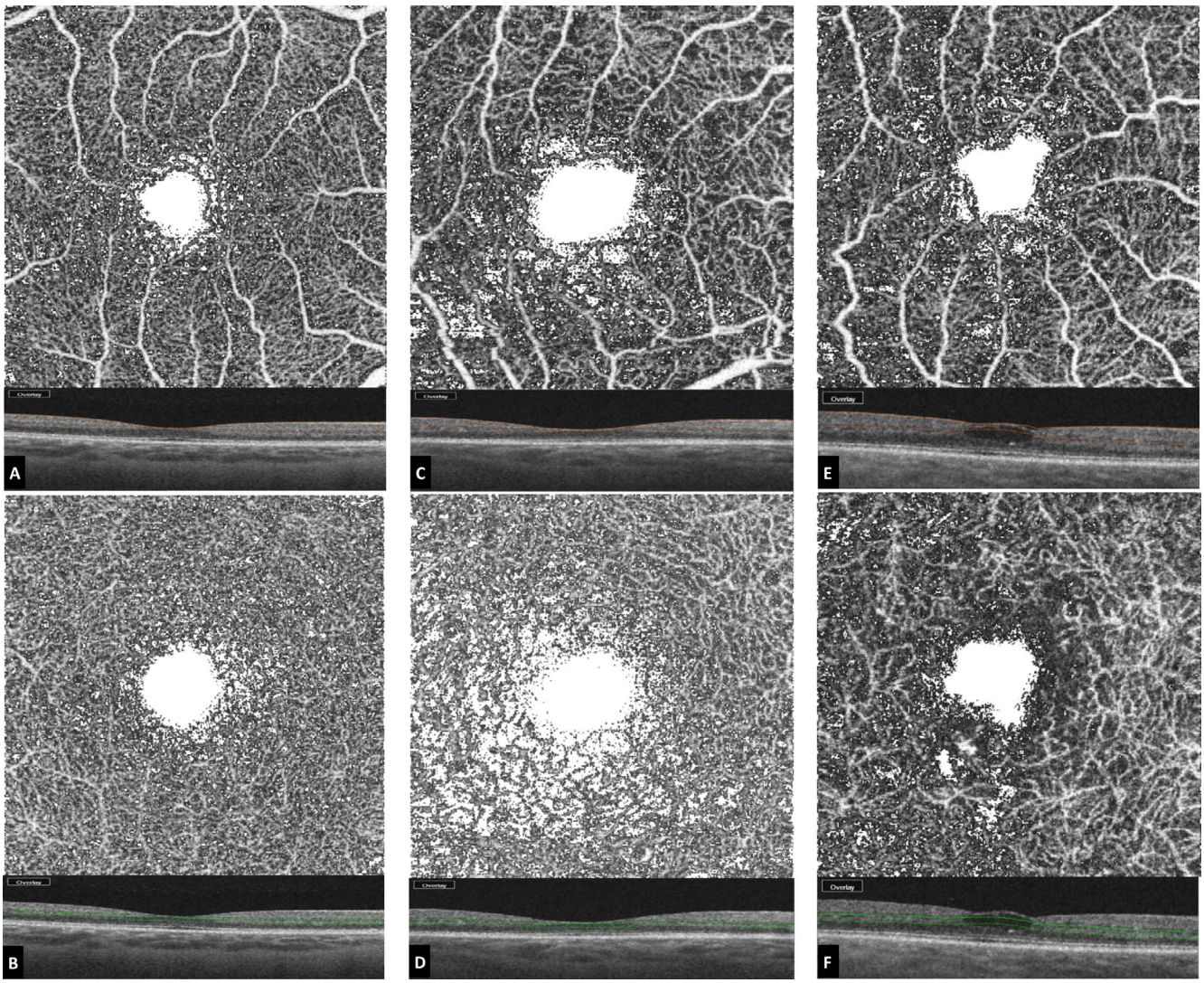

FIGURE 2. Swept-source OCT-A images of three subjects centered on the fovea. (**A**, **B**) En face projection image of the vessel density of the superficial and DRLs in a healthy individual. (**C**, **D**) Corresponding en face projection images of a patient with DR without DME. (**E**, **F**) En face OCT-A images of a patient with DR with DME.

Patients were recruited prospectively at Doheny Eye Institute, UCLA, between September and December 2015. There was no age criterion for enrollment in the study, but all subjects had a known diagnosis of diabetes mellitus, previously confirmed by laboratory testing by their primary care physician.

The subjects of the control group were deemed to be normal based on the absence of any previous ocular history, any systemic diseases, or any visual symptoms; a normal-appearing retina on clinical examination; and a normal reflectance OCT of the macula.

Swept-Source OCT-A

The images were obtained using a swept-source OCT device (DRI OCT Triton; Topcon Corporation, Tokyo, Japan) with a central wavelength of 1050 nm, an acquisition speed of 100,000 A-scans per second, and an axial and transversal resolution of 7 and 20 μm in tissue, respectively. Scans were taken from 3 × 3 mm cubes with each cube consisting of 320 clusters of four repeated B-scans centered on the fovea. En face images of the retinal vasculature were generated from the SRL and DRL, based on the automated layer segmentation performed by the OCT instrument software. The en face images of the superficial capillary network were derived from an en face slab, extending from the internal limiting membrane to the inner border of the inner nuclear layer. The en face images of the DRL were derived from a slab that extended from the inner border of the inner nuclear layer to the outer border of the inner nuclear layer.

Quantitative Measurements and Statistical Analysis

Quantitative analysis was performed using the publically available GNU Image Manipulation Program GIMP 2.8.14 (available in the public domain at http://gimp.org).

The FAZ area was defined as the area inside the central border of the capillary network, which was outlined manually for the SRL and DRL in accordance with our previously described technique (Fig. 1).[18] The graders used the "scissors" tool of the GIMP software to outline the border of the FAZ area. The software calculated the outlined area in pixels. The area then was calculated based on the 320-pixel width of the images. The measured area in pixels was converted to

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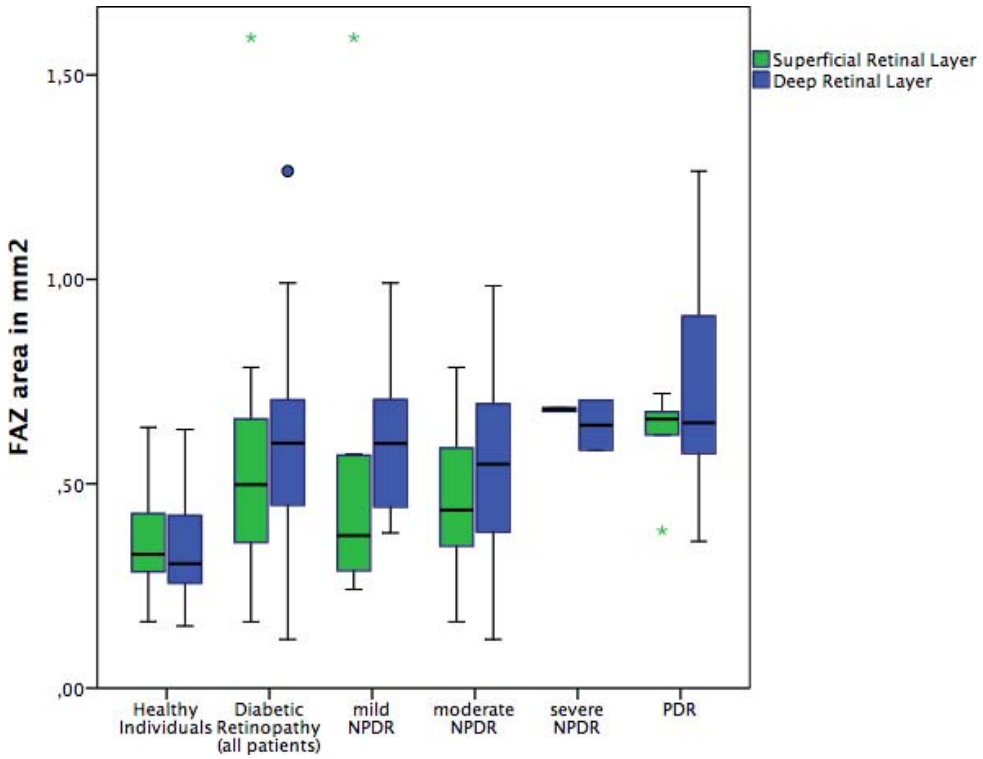

Figure 3. Boxplot showing the FAZ area in the SRLs and DRLs in healthy individuals, patients with DR and subgroups of patients with DR.

millimeters based on the scan dimensions (3 × 3-mm scan). The visible vessels in the SRL and DRL in the entire 3 × 3 macula scan were extracted using the color selection tool (Fig. 2). Vessel density (VD) was assessed as the ratio of area occupied by vessels.

The FAZ area and VD were measured by two independent, masked Doheny Image Reading Center graders. The measurements made by the main grader were used for the analysis, while those made by the second grader were used only to calculate the intergrader agreement.

The Kolmogorov-Smirnov test was used for normality. Student's *t*-test for unpaired samples was used to compare each layer of the two groups. We used SPSS statistical software version 21 (SPSS, Inc., IBM Company, Chicago, IL, USA) for statistical analysis. A P value of <0.05 was considered significant. Intraclass correlation coefficients (ICC) with 95% confidence intervals (CIs) were calculated to assess intergrader agreement.

Results

A total of 28 eyes of 18 patients with DR were included in this study. The mean age of the patients with DR was 72 years (range, 54–93), and 11 (61%) were male and 7 (39%) were female. There were 15 (83%) white and 3 (17%) Hispanic patients. Of the eyes, 10 (35.7%) had mild, 10 (35.7%) moderate, and 2 (7.1%) severe nonproliferative DR (NPDR), and 6 (21.4%) had proliferative DR (PDR). A total of 13 eyes (46.2%) had evidence of diabetic macula edema (DME) on clinical exam and/or OCT at the time of image acquisition. Among the eyes with DME, 5 had mild and 6 moderate NPDR, and 2 had PDR. Among the patients without DME, 5 had mild, 4 had moderate, and 2 had severe NPDR, and 4 had PDR.

Table 1. Mean (SD) Area of the FAZ Area in mm^2 in the SRL and DRL

	FAZ Area, mm^2		
	DR, Mean ± SD	Normal Individuals, Mean ± SD	*P* Value
SRL	0.518 ± 0.273	0.339 ± 0.118	0.003
DRL	0.615 ± 0.237	0.358 ± 0.105	<0.001

We evaluated 40 eyes of 22 healthy individuals in this study. The average age of control subjects was 39 years (range, 30–58); 9 were men and 14 were women; 16 were white, 1 black, and 4 Hispanic.

The mean (SD) FAZ area in the SRL was 0.518 (0.273) mm^2 in the DR group, and 0.339 (0.118) mm^2 in the control group ($P = 0.003$; Fig. 3, Table 1). The mean (SD) FAZ area in the DRL was 0.615 (0.237) mm^2 in the DR group and 0.358 (0.105) mm^2 in healthy individuals ($P < 0.001$; Fig. 3, Table 1). After dividing the groups based on their DR stage, the mean VD (SD) and mean FAZ area (SD) are shown in Table 2. Comparing those subgroups to healthy individuals, there was a statistically significant difference in the FAZ area in the SRL in patients with severe NPDR and PDR ($P = 0.301$ in mild, $P = 0.199$ in moderate, and $P < 0.001$ in severe NPDR, and $P < 0.001$ in the PDR group). In the DRL, all subgroups showed a statistically significant difference in the FAZ area ($P < 0.001$ in mild, $P = 0.029$ in moderate, and $P = 0.001$ in severe NPDR, and $P = 0.027$ in the PDR group). The mean difference between the two graders was 0.012 ± 0.030 mm^2 for the SRL and 0.011 ± 0.041 mm^2 for the DRL. The ICC was 0.997 (95% CI, 0.993–0.999) and 0.992 (95% CI, 0.983–0.996) for the SRL and DRL.

The mean VD was statistically significantly lower in patients with DR in the SRL and DRL. In the SRL, the mean (SD) VD ratio was 0.567 (0.097) in patients with DR and 0.709 (0.038) in normal individuals ($P < 0.001$; Fig. 4, Table 3). In the DRL, the mean (SD) ratio was 0.668 (0.099) and 0.714 (0.049), respectively, ($P = 0.028$; Fig. 4, Table 3). After dividing the

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Table 2. Mean (SD) of the VD (Ratio) and FAZ Area in the SRL and DRL in the Different Stages of DR

	Mild NPDR		Moderate NPDR		Severe NPDR		PDR	
	Mean ± SD	*P* Value	Mean ± SD	*P* Value	Mean ± SD	*P* Value	Mean ± SD	*P* Value
FAZ in mm^2								
SRL	0.498 ± 0.401	0.301	0.444 ± 0.191	0.199	0.681 ± 0.006	<0.001	0.619 ± 0.119	<0.001
DRL	0.608 ± 0.187	<0.001	0.546 ± 0.250	0.029	0.643 ± 0.086	0.001	0.734 ± 0.314	0.027
VD, ratio								
SRL	0.591 ± 0.097	0.004	0.566 ± 0.114	0.003	0.589 ± 0.043	0.148	0.519 ± 0.081	0.002
DRL	0.699 ± 0.076	0.544	0.649 ± 0.118	0.122	0.704 ± 0.079	0.773	0.636 ± 0.017	0.136

groups based on their DR stage, the mean VD (SD) and mean FAZ area (SD) are shown in Table 2. Comparing those subgroups to healthy individuals, there was a statistically significant difference in the VD in the SRL in patients with mild or moderate NPDR as well as PDR (*P* value 0.004 in mild, 0.003 in moderate, and 0.148 in severe NPDR, and 0.002 in the PDR group). There was no statistically significant difference in the DRL compared to healthy individuals. The mean difference between the two graders was 0.010 ± 0.032 for the SRL and 0.015 ± 0.059 for the DRL. The ICC was 0.972 (95% CI, 0.941–0.987) and 0.889 (95% CI, 0.764–0.948) for the SRL and DRL.

Dividing the cohort with regard to the presence of DME (46.42%, 13 eyes) at time of exam, we found a statistically significant difference in the VD in the DRL compared to healthy individuals. The mean (SD) VD in the group of patients with DME was 0.534 (0.088) and 0.617 (0.070) in the SRL and DRL, respectively. In the patients without DME, the VD was 0.594 (0.099) and 0.706 (0.102) in the SRL and DRL, respectively (Table 4). The mean VD in patients with DME was statistically significantly lower than patients without DME.

Discussion

This study evaluated the FAZ area and VD of the macular capillary network, using swept-source OCT-A in patients with DR. We observed a statistically significant enlargement of the FAZ and a lower VD of the capillary network in the SRL and DRL compared to healthy individuals.

Previous studies have used FA to identify enlargement of the FAZ area in patients with DR. In these studies, there was a strong correlation between the FAZ area and severity of the capillary nonperfusion.[12,23] Others defined the enlargement of the FAZ area as an indicator of DR progression.[24] Using OCT-A, our study showed a statistically significant enlargement in patients with DR at the SRL and DRL, suggesting that progressive nonperfusion in these patients was not limited to a particular layer. These results are consistent with other previous reports.[25–27] Furthermore, we measured the VD in each layer of the retina. As seen in Table 3, there was a statistically significant decrease in VD in the SRL and DRL between patients with DR and healthy individuals. Although both layers were affected, a more consistent and severe decrease in VD was observed in the SRL. This observation is

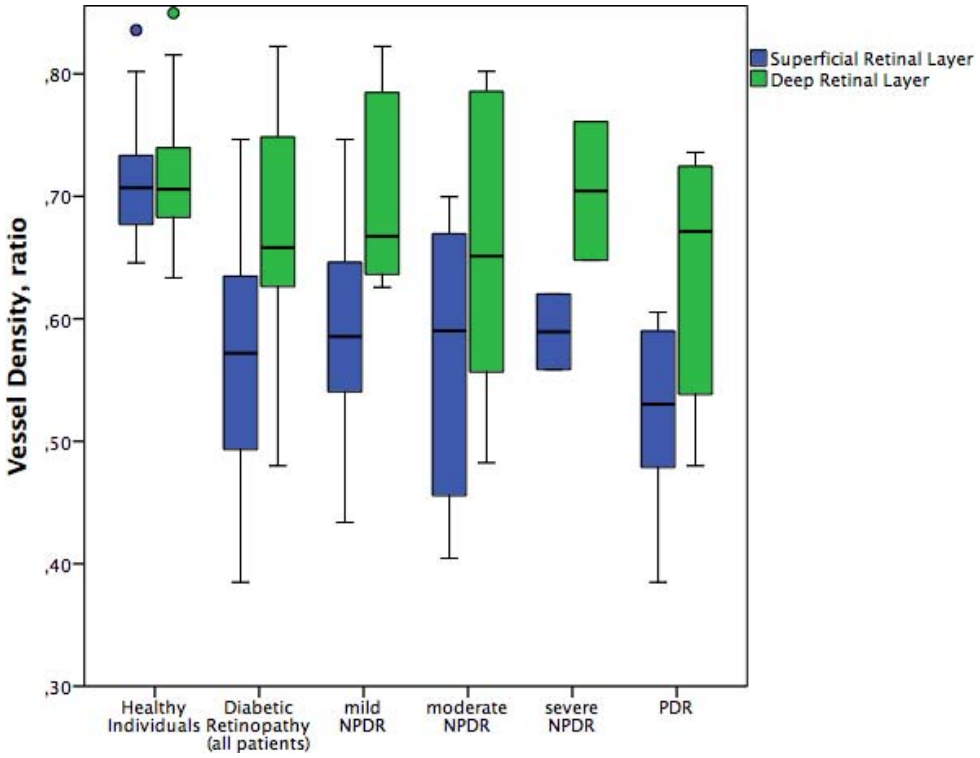

Figure 4. Boxplot showing the VD ratio as the retinal area occupied by vessels in the SRLs and DRLs in healthy individuals, those with DR and subgroups of patients with DR.

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Table 3. Mean (SD) of the VD (Ratio) in the SRL and DRL

	VD, Ratio		
	DR, Mean ± SD	Normal Individuals, Mean ± SD	*P* Value
SRL	0.567 ± 0.097	0.709 ± 0.038	<0.001
DRL	0.668 ± 0.099	0.714 ± 0.049	0.028

consistent with a recent study demonstrating that the macular superficial capillary network in diabetics has more extensive nonperfused areas than the DRL.[21] Considering the subgroups of patients with DR, the VD in the SRL in patients with mild or moderate NPDR and PDR was statistically significantly lower compared to normal individuals, whereas the VD in the DRL did not show a statistically significant difference. Upon further subdivision of the DR group based on the presence of DME, we observed a reduced VD in the DRL in eyes with DME compared to those without DME. We speculate that this difference may be related to the presence of cystoid changes, which appear as areas of no-flow and are included in the nonperfusion computation. Such an effect has been demonstrated by de Carlo et al.[28] However, if the vessels are simply displaced by the cysts and not actually lost, creating a thicker en face slab that includes the entire cyst and surrounding tissue may allow this question to be explored further.

The variability in capillary density measurements was greater among the eyes with DR compared to the normal eyes. This likely reflects, in part, the varying severities of retinopathy within the diabetic cohort. Patients with mild-to-moderate NPDR seemed to have a smaller FAZ and a higher capillary density compared to patients with more severe retinopathy; however, our study was not powered to make these comparisons.

Despite the relatively small cohort size, the high repeatability of our measurements gives us confidence that the differences between the diabetic and normal eyes are reliable. The repeatability between graders for FAZ area and capillary density was excellent with an ICC of 0.997 and 0.992 for the FAZ and 0.972 and 0.889 for the VD, and a coefficient of variation of 0.01.

In contrast to previous studies, we used a swept-source OCT-A with a light source centered at 1050 nm, which can penetrate tissues to a greater extent with less sensitivity roll-off with depth. This potentially could be an advantage for evaluating the microcirculation in the setting of markedly thickened or edematous retinas.

In addition to the small number of subjects with specific severities of retinopathy, our study has other limitations, which should be considered when assessing our findings. First, our measurements were based on a single session. It is not known whether the result would have differed if we repeated the OCT-A scans a few days later. We have, however, previously studied the repeatability of OCT-A measurements between sessions, and we have found them to have a high degree of reproducibility. Another limitation of this study is that the number of normal subjects was small, and their mean age was younger than that of the patients with DR. An additional limitation of the present study is the small field of view (3 × 3 mm). A larger field of view using OCT-A may allow further investigation, such as the correlation between FAZ area and extent of ischemia in the periphery. Although scans with a larger field of view may be obtained, it is at the expense of lateral resolution, which may impair accurate capillary measurements. Future advances allowing montaging of multiple scan acquisitions may partially address this limitation. Another major limitation of current OCT-A technology is the accuracy of segmentation algorithms, particularly in diseases featuring significant disruption of the retinal layers. Instrument software does allow correction of these segmentation errors, but such correction would be impractical when hundreds of individual B-scans would need to be corrected per case. Further improvement of the automated segmentation or the development of more efficient semiautomated correction tools is required. Another limitation of existing OCT-A devices is the background noise, which could affect the quantitative measurements as it could be mistaken as flow and mistakenly included in the VD measurement.

Table 4. Mean (SD) of the VD (Ratio) in the SRL and DRL in Patients With/Without DME

	VD, Ratio		
	With DME, Mean ± SD	Without DME, Mean ± SD	*P* Value
SRL	0.534 ± 0.088	0.594 ± 0.099	>0.05
DRL	0.617 ± 0.070	0.706 ± 0.102	0.014

In summary, in this study we were able to use swept-source OCT-A to reliably demonstrate an enlargement of the FAZ area and a reduction in retinal capillary density in the SRL and DRL in eyes with DR. Our findings highlight the potential role of OCT-A in monitoring and quantifying retinal vascular alterations in diabetes.[29]

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