

# A Study of the Association Between Retinal Vessel Geometry and Optical Coherence Tomography Angiography Metrics in Diabetic Retinopathy

Ji Min Choi,<sup>1</sup> Seong Mi Kim,<sup>2</sup> Young Hwan Bae,<sup>3</sup> and Dae Joong Ma<sup>3</sup>

<sup>1</sup>Department of Internal Medicine, Healthcare Research Institute, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Republic of Korea

<sup>2</sup>Department of Ophthalmology, Jeju National University College of Medicine, Jeju-si, Jeju-do, Republic of Korea

<sup>3</sup>Department of Ophthalmology, Hallym University Kangnam Sacred Heart Hospital, Seoul, Republic of Korea

Correspondence: Dae Joong Ma, Department of Ophthalmology, Hallym University Kangnam Sacred Heart Hospital, 1, Singil-ro, Yeongdeungpo-gu, Seoul 07441, Republic of Korea; [daejoongma@hallym.or.kr](mailto:daejoongma@hallym.or.kr).

JMC and SMK contributed equally to the work presented here and should therefore be regarded as equivalent authors.

**Received:** December 23, 2020

**Accepted:** July 21, 2021

**Published:** October 18, 2021

Citation: Choi JM, Kim SM, Bae YH, Ma DJ. A study of the association between retinal vessel geometry and optical coherence tomography angiography metrics in diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 2021;62(13):14. <https://doi.org/10.1167/iovs.62.13.14>

**PURPOSE.** The purpose of this study was to investigate whether optical coherence tomography angiography (OCTA) metrics are related to retinal vessel geometry parameters in diabetic retinopathy (DR).

**METHODS.** In total, 119 eyes (119 patients) were included in this retrospective cross-sectional study. Retinal vessel geometry parameters were analyzed using semi-automated software. OCTA metrics were analyzed using automated manufacturer-provided algorithms. Associations between the severity of DR and retinal vessel geometry parameters and OCTA metrics were evaluated. Multivariable regression analyses were performed to evaluate associations between retinal vessel geometry parameters and OCTA metrics after adjusting for clinical characteristics and DR severity.

**RESULTS.** DR severity was negatively associated with the following: arteriole-venular ratio ( $P = 0.039$ ), arteriolar network fractal dimension (FDa;  $P = 0.003$ ), arteriolar junctional exponent deviation ( $P = 0.037$ ), venular junctional exponent deviation ( $P = 0.036$ ), vessel area density (VAD) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP;  $P < 0.001$ , both), vessel length density (VLD) of the SCP and DCP ( $P < 0.001$ , both), and foveal avascular zone (FAZ) circularity ( $P < 0.001$ ). DR severity was positively associated with the central retinal venular equivalent caliber ( $P = 0.005$ ), arteriolar branching coefficient (BCa;  $P = 0.010$ ), venular branching coefficient ( $P = 0.007$ ), and FAZ size ( $P = 0.002$ ). In multivariable regression analyses, the following retinal vessel geometry parameters and OCTA metrics were associated: FDa with VAD of the SCP ( $\beta = 0.40$ ,  $P < 0.001$ ), FDa with VLD of the SCP ( $\beta = 0.01$ ,  $P < 0.001$ ), and BCa with FAZ circularity ( $\beta = -1.02$ ,  $P = 0.001$ ).

**CONCLUSIONS.** In DR, changes in retinal arteriolar geometry parameters were significantly associated with OCTA metrics, which reflect DR pathophysiology.

**Keywords:** diabetic retinopathy (DR), geometry, optical coherence tomography angiography (OCTA), retina, vasculature

Diabetic retinopathy (DR) is the leading cause of visual impairment and blindness worldwide, especially among people of working age.<sup>1</sup> Chronic diabetes mellitus (DM)-related hyperglycemia levels lead to macrovascular and microvascular complications. Macrovascular complications are mainly due to atherosclerotic narrowing of large arteries and veins, leading to cardiovascular, cerebrovascular, and peripheral arterial diseases.<sup>2</sup> Microvascular complications occur in small vessels, such as arterioles, venules, and capillaries, leading to diabetic microangiopathy, diabetic nephropathy, diabetic neuropathy, and DR.<sup>3</sup> DR is characterized as increased vascular permeability, vascular occlusions, ischemia, and subsequent angiogenesis, which starts with retinal capillary damage.<sup>4</sup> Clinical evaluation of DR is mainly based on a fundus examination; however, the severity of DR is assessed using semiquantitative grading systems based

on the presence or absence of retinal lesions, including microaneurysms, hemorrhages, intraretinal microvascular abnormalities, venous beading/loops/reduplications, and neovascularization. These signs only partially reflect the microvascular changes associated with DR, especially those of retinal capillaries.

Retinal vessel geometry parameters, such as retinal arteriolar or venular diameters, tortuosity, fractal dimensions, and branching angles, can be derived quantitatively from the semi-automated analysis of fundus photographs.<sup>5</sup> Several studies have reported that changes in retinal vessel geometry parameters were associated with both retinal and systemic DM-related complications, including diabetic nephropathy and cardiovascular diseases.<sup>6-8</sup> However, possible mechanisms underlying the retinal vessel geometric changes involved in DM-related complications are

not fully understood. This may be because retinal vessel geometric changes only reflect changes to retinal arterioles and venules and not those to retinal capillaries in which microvascular complications associated with DR largely take place.

Optical coherence tomography angiography (OCTA) is a recently developed technology that enables noninvasive, rapid, and accurate visualization of the retinal microvasculature, including the capillaries.<sup>9</sup> In this study, we hypothesized that alterations of retinal arteriolar and venular geometry in DR would be associated with retinal capillary changes. We aimed to evaluate geometric changes to retinal arterioles and venules and those to retinal capillaries in DR using fundus photography and OCTA. Moreover, we aimed to investigate the associations between retinal vessel geometry parameters and OCTA metrics that reflect retinal capillary changes, which reflect DR pathophysiology.

## RESEARCH DESIGN AND METHODS

### Ethical Approval

The study was approved by the Institutional Review Board (IRB) of Jeju National University Hospital (IRB approval number: 2019-07-002) and was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was waived by the IRB.

### Study Population

This single-center retrospective cross-sectional study comprised all consecutive patients who had been referred for DR screening and who had undergone digital retinal photography and OCTA scans between March 2019 and March 2020 at Jeju National University Hospital in Jeju-si, Jeju-do, Republic of Korea. Inclusion criteria comprised referred patients ranging from those with no DR to nonproliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR). Exclusion criteria comprised patients who had previously undergone laser photocoagulation, intravitreal injections, and intraocular surgeries, except for cataract surgery. We also excluded patients with concurrent retinal diseases other than DR, media opacity adversely affecting fundus photography or OCTA acquisition, and those with poor image quality.

### General Examination and Laboratory Tests

Patients were referred from the Endocrinology Center, where a thorough evaluation for DM had been performed. We retrospectively collated patient information from related medical records, including the presence of hypertension, the duration of DM, and hemoglobin A1c levels.

### Ophthalmic Examination

All patients underwent a complete ophthalmological examination that included best-corrected visual acuity measurement using a Snellen chart, tonometry, slit lamp biomicroscopy, fundus photography, and OCTA scans. Eyes were classified based on the International Clinical Classification of DR, which includes five stages, as described previously.<sup>1</sup>

## Retinal Vessel Geometry

Digital retinal photographs were obtained using a Visucam NM/FA fundus camera (Carl Zeiss Meditec, Inc., Dublin, CA, USA) in 45-degree mode centered on the optic disc. The eye with better retinal photograph quality was included in the study. Retinal vessel geometry was analyzed by two independent trained graders (D.J.M. and J.M.C) using semi-automated software (Singapore I Vessel Assessment [SIVA] – cloud-based version, National University of Singapore, Singapore) in accordance with the developer's protocol. Briefly, the program automatically traced and identified all vessels (arterioles and venules) and generated a skeleton image of the retinal microvasculature. In cases of optic disc detection error, a vascular tracing error, or misidentification of a vessel, a grader manually relocated the optic disc, corrected the erroneous vascular tracing, and reidentified the vessels. All graders were masked to the data during manual correction. SIVA then automatically generated geometric parameters of retinal vessels. Data concerning retinal vessel geometry parameters included in this study and their descriptions are shown in Table 1. The ring-shaped retinal area between the second and the fifth optic disc radii from the optic disc center was analyzed (Zone C, Fig. 1).

## OCTA Metrics

The OCTA scans used in this study were obtained using a PLEX Elite 9000 (Carl Zeiss Meditec) with a 6 × 6 mm field of view that covered the perifoveal region of the retina (Fig. 2). Quantitative analysis of the OCTA images, including vessel area density (VAD), vessel length density (VLD), and foveal avascular zone (FAZ) metrics, such as circularity index and size, was performed using the ARI Network Test Algorithms provided by the manufacturer (available at <https://arinetworkhub.com/>). VAD was defined as the total area of perfused vasculature per unit area, ranging from 0.00 (no perfusion) to 1.00 (fully perfused), which was evaluated in the superficial capillary plexus (SCP; VAD<sub>SCP</sub>) and the deep capillary plexus (DCP; VAD<sub>DCP</sub>). VLD was defined as the total length of perfused vasculature per unit area in a region of measurement (mm<sup>-1</sup>), evaluated in the SCP (VLD<sub>SCP</sub>) and the DCP (VLD<sub>DCP</sub>). The FAZ circularity index was calculated as a function of the perimeter and the area of a shape, which reflects a measure of the compactness of a shape relative to a circle, ranging from 0.0 (irregular shape) to 1.0 (a circular shape). Data from eyes with a central macular thickness > 300 μm were excluded from the analysis due to the high risk of erroneous segmentation.

## Statistical Analysis

Data are expressed as mean ± standard deviation or as numbers and percentages. Snellen best corrected visual acuity measurements were converted to logMAR units prior to statistical analysis. The Kruskal-Wallis test, followed by the Mann-Whitney *U* test with Bonferroni correction, was used to assess associations between DR severity and clinical characteristics. Intergrader reliability estimates of retinal vessel geometry parameters were assessed using the intraclass correlation coefficient (ICC). The mean of the retinal vessel geometry parameters evaluated by the two graders was used in the analysis.

Associations between DR severity and retinal vessel geometry parameters and OCTA metrics were assessed using

TABLE 1. Data Describing the 13 Retinal Vascular Parameters Measured for Each Retinal Photograph<sup>5</sup>

Parameter	Description
Central retinal arteriolar equivalent caliber (CRAE)	Estimated width of central retinal artery or vein derived from the summary measures of the six largest arteriole or venule using the revised Knudtson-Parr-Hubbard formula. <sup>25</sup>
Central retinal venular equivalent caliber (CVAE)	
Arteriole-venular ratio (AVR)	The ratio of CRAE with respect to CRVE.
Fractal dimension of arteriolar network (FDa)	Global measures that summarize the branching complexity of the retinal vascular tree.
Fractal dimension of venular network (FDv)	
Arteriolar curvature tortuosity (TORTa)	Integral of the curvature squared along the sample vessel segments path, normalized by the total path length.
Venular curvature tortuosity (TORTv)	
Arteriolar branching coefficient (BCa)	Ratio of the two daughter branching vessels width ( $D_1$ and $D_2$ ) to trunk vessel width ( $D_0$ ): $(D_1^2 + D_2^2)/D_0^2$
Venular branching coefficient (BCv)	
Arteriolar junctional exponent deviation (JEa)	Deviation of the junctional exponent ( $x$ ) from the Murray's law prediction: $D_1^x + D_2^x = D_0^x$ , $x = 3.0$
Venular junctional exponent deviation (JEv)	

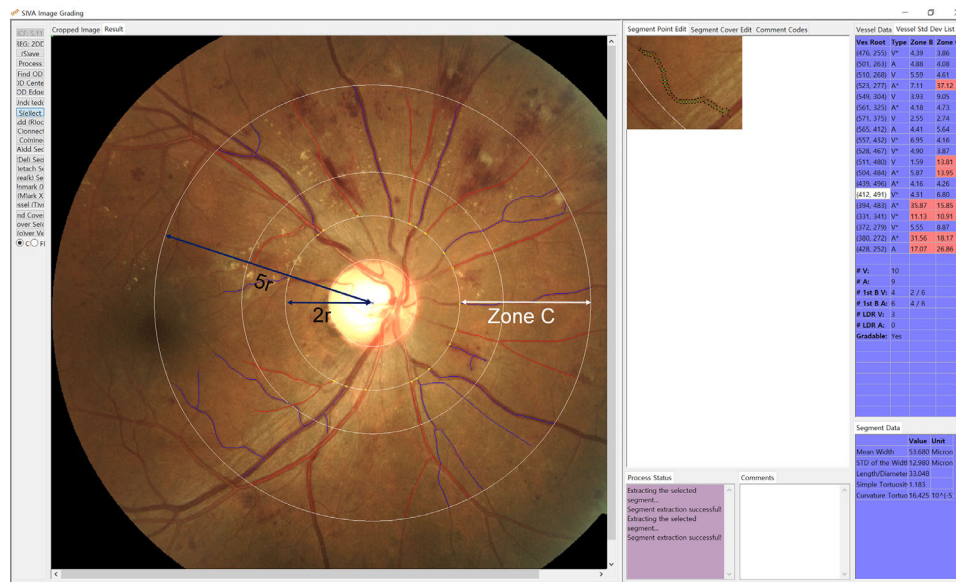


FIGURE 1. A screenshot of retinal vessel geometry analysis using Singapore “I” Vessel Assessment (SIVA) cloud-based version. Arterioles are marked in red and venules in blue. The white arrow indicates zone C, which is the ring-shaped area between second and the fifth optic disc radii from the optic disc center.

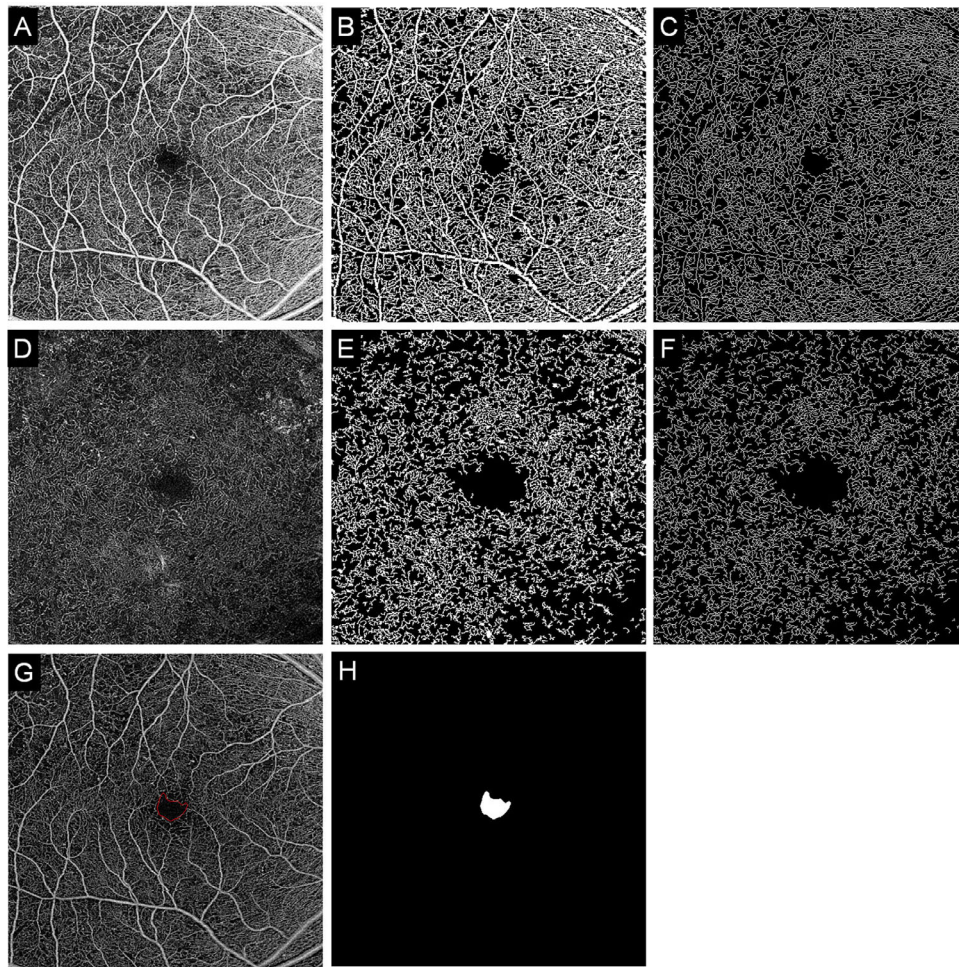
Pearson’s correlation analysis. Exploratory univariable linear regression analyses were performed to evaluate associations between retinal vessel geometry parameters and OCTA metrics that showed an association with DR severity. All variables with  $P$  values  $< 0.05$  in the univariable analysis were included in the multivariable linear regression analyses with stepwise variable selection. Multivariable regression analyses were performed after adjusting for clinical characteristics and DR severity and corrected for multiple testing using a Benjamini–Hochberg correction.

A  $P$  value  $< 0.05$  was considered statistically significant. Statistical analyses were performed using IBM SPSS version 22.0 Statistics for Windows (IBM Corp., Armonk, NY, USA) and MedCalc version 12.3.0.0 (MedCalc Software, Mariakerke, Belgium) software.

## RESULTS

A total of 59 right eyes (49.6%) and 60 left eyes (50.4%) from 119 patients were included in the study. Data concerning the clinical characteristics of patients at each DR severity level are summarized in Table 2. Eyes were classified as no DR ( $n = 40$ ), mild NPDR ( $n = 22$ ), moderate NPDR ( $n = 22$ ), severe NPDR ( $n = 28$ ), and PDR ( $n = 7$ ). No significant differences were observed in terms of age, sex, the presence of hypertension, hemoglobin A1c level, visual acuity, or central macular thickness among the DR severity groups. However, post hoc analysis indicated that patients without DR (no DR group) had a shorter DM duration compared with those in the severe NPDR group ( $P = 0.022$ ).





**FIGURE 2.** Automatically generated images for the measurement of optical coherence tomography angiography (OCTA) metrics by ARI Network. (A) En face OCTA image of the superficial capillary plexus (SCP) of the eye shown in Figure 1. (B) Binarized image of the SCP for the calculation of vessel area density. (C) Skeletonized binarized image of the SCP for the calculation of vessel length density. (D) Projection removed en face OCT image of the deep capillary plexus (DCP) of the same eye. (E) Binarized image of the DCP. (F) Skeletonized binarized image of the DCP. (G) Automatically drawn SCP foveal avascular zone (FAZ) boundary for the calculation of circularity. (H) FAZ area map for the calculation of size.

**TABLE 2.** Data of Clinical Characteristics at Each Diabetic Retinopathy Severity Level

	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	<i>P</i> Value
Age (years)	57.97 ± 15.29	56.55 ± 17.37	59.31 ± 16.18	60.64 ± 10.80	48.69 ± 15.22	0.467*
Male: female (n)	22:18	14:8	17:5	21:7	4:3	0.323†
Hypertension (%)	42.5	40.9	40.9	46.4	42.9	0.995†
Duration of DM (years)	7.24 ± 9.61	10.18 ± 5.23	10.75 ± 9.38	12.59 ± 9.22	14.57 ± 8.79	0.008*
HbA1c (%)	9.35 ± 3.10	8.30 ± 1.96	9.52 ± 2.61	8.48 ± 1.95	10.25 ± 4.13	0.524*
Visual acuity (logMAR)	0.13 ± 0.18	0.04 ± 0.09	0.12 ± 0.17	0.12 ± 0.14	0.12 ± 0.13	0.084*
Central macular thickness (μm)	253.28 ± 20.97	267.55 ± 19.95	264.14 ± 30.74	267.96 ± 37.35	261.50 ± 53.95	0.179*

DM, diabetes mellitus; HbA1c, hemoglobin A1c; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

\* Kruskal-Wallis test.

† Chi-square test.

### Associations Between DR Severity and Retinal Vessel Geometry Parameters and OCTA Metrics

Intergrader reliability estimates of retinal vessel geometry parameters are shown in Supplementary Table S2. Intergrader reliability was high for all the retinal vessel geometry

parameters, with ICCs  $\geq 0.80$ . Mean retinal vessel geometry parameters evaluated by the two graders, shown in Table 3, were used in further analysis.

DR severity was negatively associated with the arteriole-to-venule ratio (AVR;  $r = -0.19$ ,  $P = 0.039$ ), the fractal dimension of the arteriolar network (FDA;  $r = -0.27$ ,

TABLE 3. Data of Comparison and Correlation of Retinal Arteriolar and Venular Geometry Parameters According to Diabetic Retinopathy Severity

	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Correlation* r Value	P Value
GRAE	132.18 ± 9.70	132.41 ± 10.02	132.38 ± 10.51	134.32 ± 8.56	137.20 ± 13.60	0.12	0.212
CRVE	193.55 ± 15.24	187.49 ± 19.35	201.55 ± 22.11	201.50 ± 16.38	207.61 ± 14.70	0.26	0.005
AVR	0.69 ± 0.05	0.71 ± 0.07	0.66 ± 0.04	0.67 ± 0.05	0.66 ± 0.04	-0.19	0.039
FDa	1.21 ± 0.04	1.22 ± 0.04	1.19 ± 0.05	1.18 ± 0.05	1.18 ± 0.06	-0.27	0.003
FDv	1.22 ± 0.04	1.21 ± 0.04	1.20 ± 0.05	1.21 ± 0.06	1.20 ± 0.04	-0.08	0.383
TORTa	$7.85 \times 10^{-5} \pm 1.65 \times 10^{-5}$	$7.79 \times 10^{-5} \pm 9.87 \times 10^{-6}$	$7.87 \times 10^{-5} \pm 1.65 \times 10^{-5}$	$7.65 \times 10^{-5} \pm 2.08 \times 10^{-5}$	$7.80 \times 10^{-5} \pm 1.62 \times 10^{-5}$	-0.03	0.755
TORTv	$8.90 \times 10^{-5} \pm 1.63 \times 10^{-5}$	$8.47 \times 10^{-5} \pm 1.51 \times 10^{-5}$	$8.64 \times 10^{-5} \pm 1.21 \times 10^{-5}$	$9.20 \times 10^{-5} \pm 1.70 \times 10^{-5}$	$9.92 \times 10^{-5} \pm 1.87 \times 10^{-5}$	0.13	0.159
BCa	1.60 ± 0.28	1.69 ± 0.36	1.57 ± 0.49	1.78 ± 0.63	1.85 ± 0.40	0.24	0.010
BCv	1.31 ± 0.20	1.38 ± 0.17	1.35 ± 0.28	1.49 ± 0.26	1.40 ± 0.21	0.24	0.007
JEa	-0.49 ± 0.34	-0.57 ± 0.34	-0.59 ± 0.34	-0.69 ± 0.37	-0.65 ± 0.62	-0.20	0.037
JEv	-0.16 ± 0.34	-0.28 ± 0.33	-0.14 ± 0.44	-0.40 ± 0.29	-0.28 ± 0.23	-0.19	0.036

AVR, arteriole-venular ratio; BCa, arteriolar branching coefficient; BCv, venular branching coefficient; CRAE, central retinal arteriolar equivalent caliber; CRVE, central retinal venular equivalent caliber; FDa, fractal dimension of arteriolar network; FDv, fractal dimension of venular network; JEa, junctional exponent deviation for arterioles; JEv, junctional exponent deviation for venules; TORTa, arteriolar curvature tortuosity; TORTv, venular curvature tortuosity.

**TABLE 4.** Data of Comparison and Correlation of Optical Coherence Tomography Angiography Metrics According to Diabetic Retinopathy Severity

		No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	Correlation* <i>r</i> Value	P-Value
<b>VAD</b>	<b>SCP</b>	0.42 ± 0.03	0.41 ± 0.04	0.39 ± 0.03	0.39 ± 0.04	0.35 ± 0.04	-0.46	<0.001
	<b>DCP</b>	0.26 ± 0.08	0.23 ± 0.09	0.19 ± 0.07	0.20 ± 0.06	0.14 ± 0.07	-0.41	<0.001
<b>VLD (mm<sup>-1</sup>)</b>	<b>SCP</b>	19.14 ± 1.51	18.63 ± 2.15	17.48 ± 1.22	17.33 ± 1.84	17.53 ± 4.14	-0.38	<0.001
	<b>DCP</b>	12.97 ± 3.68	11.43 ± 4.12	9.29 ± 3.23	9.83 ± 2.73	8.08 ± 5.70	-0.39	<0.001
<b>FAZ</b>	<b>Circularity</b>	0.71 ± 0.09	0.69 ± 0.13	0.68 ± 0.07	0.64 ± 0.13	0.51 ± 0.27	-0.34	<0.001
	<b>Size (mm<sup>2</sup>)</b>	0.32 ± 0.14	0.31 ± 0.19	0.35 ± 0.11	0.43 ± 0.18	1.29 ± 1.69	0.29	0.002

DCP, deep capillary plexus; DR, diabetic retinopathy; FAZ, foveal avascular zone; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SCP, superficial capillary plexus; VAD, vessel area density; VLD, vessel length density.

**TABLE 5.** Data Depicting Relation of the Diabetic Retinopathy Severity Depending Retinal Vessel Geometry Parameters with Optical Coherence Tomography Angiography Metrics

			VAD		VLD (mm <sup>-1</sup> )		FAZ	
			SCP	DCP	SCP	DCP	Circularity	Size (mm <sup>2</sup> )
<b>CRVE</b>	Univariable	<i>β</i>	13.89	11.44	0.23	0.28	-11.07	7.74
		<i>P</i> value	0.758	0.607	0.801	0.538	0.440	0.071
<b>AVR</b>	Univariable	<i>β</i>	-0.03	-0.02	-2.10 × 10 <sup>-3</sup>	-9.11 × 10 <sup>-4</sup>	0.05	-2.86 × 10 <sup>-3</sup>
		<i>P</i> value	0.809	0.725	0.451	0.519	0.299	0.830
<b>FDa</b>	Univariable	<i>β</i>	0.40	0.10	0.01	2.23 × 10 <sup>-3</sup>	0.10	-0.02
		<i>P</i> value	<0.001	0.065	<0.001	0.045	0.007	0.036
	Multivariable*	<i>β</i>	0.40	-	0.01	0.06	0.17	-0.13
		<i>P</i> value	<0.001	-	<0.001	0.525	0.079	0.149
<b>BCa</b>	Univariable	<i>β</i>	-0.99	-0.38	-0.01	-0.01	-1.02	0.15
		<i>P</i> value	0.302	0.420	0.569	0.515	0.001	0.096
	Multivariable*	<i>β</i>	-	-	-	-	-1.02	-
		<i>P</i> value	-	-	-	-	0.001	-
<b>BCv</b>	Univariable	<i>β</i>	-0.89	-0.04	-0.01	7.42 × 10 <sup>-4</sup>	-0.143	0.01
		<i>P</i> value	0.126	0.896	0.603	0.900	0.439	0.856
<b>JEa</b>	Univariable	<i>β</i>	1.15	0.47	0.01	0.01	0.70	-0.16
		<i>P</i> value	0.193	0.287	0.471	0.365	0.013	0.058
	Multivariable*	<i>β</i>	-	-	-	-	0.18	-
		<i>P</i> value	-	-	-	-	0.076	-
<b>JEv</b>	Univariable	<i>β</i>	0.87	0.09	0.01	1.18 × 10 <sup>-3</sup>	0.237	-0.04
		<i>P</i> value	0.329	0.837	0.588	0.895	0.402	0.620

AVR, arteriole-venular ratio; BCa, arteriolar branching coefficient; BCv, venular branching coefficient; CRVE, central retinal venular equivalent caliber; DCP, deep capillary plexus; FAZ, foveal avascular zone; FDa, fractal dimension of arteriolar network; JEa, junctional exponent deviation for arterioles; JEv, junctional exponent deviation for venules; SCP, superficial capillary plexus; VAD, vessel area density; VLD, vessel length density.

\*Adjusted for age, sex, diabetic retinopathy severity, and presence of hypertension.

$P = 0.003$ ), the junctional exponent deviation for arterioles (JEa;  $r = -0.20$ ,  $P = 0.037$ ), and the junctional exponent deviation for venules ( $r = -0.19$ ,  $P = 0.036$ ; see Table 3). In contrast, DR severity was positively associated with the central retinal venular equivalent caliber ( $r = 0.26$ ,  $P = 0.005$ ), the arteriolar branching coefficient (BCa;  $r = 0.24$ ,  $P = 0.010$ ), and the venular branching coefficient ( $r = 0.24$ ,  $P = 0.007$ ).

Analyses of OCTA metrics showed that DR severity was negatively associated with VAD<sub>SCP</sub> ( $r = -0.46$ ,  $P < 0.001$ ), VAD<sub>DCP</sub> ( $r = -0.41$ ,  $P < 0.001$ ), VLD<sub>SCP</sub> ( $r = -0.38$ ,  $P < 0.001$ ), VLD<sub>DCP</sub> ( $r = -0.39$ ,  $P < 0.001$ ), and FAZ circularity ( $r = -0.34$ ,  $P < 0.001$ ; Table 4), but positively correlated with FAZ size ( $r = 0.29$ ,  $P = 0.002$ ).

### Associations Between Retinal Vessel Geometry Parameters and OCTA Metrics

Only retinal vessel geometry parameters and OCTA metrics that showed a statistically significant association with DR

severity were included in the exploratory univariable linear regression analyses (Table 5). In the univariable linear regression analysis, we found an association between the following retinal vessel geometry parameters and OCTA metrics: (i) FDa and VAD<sub>SCP</sub> ( $\beta = 0.40$ ,  $P < 0.001$ ), VLD<sub>SCP</sub> ( $\beta = 0.01$ ,  $P < 0.001$ ), VLD<sub>DCP</sub> ( $\beta = 2.23 \times 10^{-3}$ ,  $P = 0.045$ ), FAZ circularity ( $\beta = 0.10$ ,  $P = 0.007$ ), and FAZ size ( $\beta = -0.02$ ,  $P = 0.036$ ); (ii) BCa and FAZ circularity ( $\beta = -1.02$ ,  $P = 0.001$ ), and (iii) JEa and FAZ circularity ( $\beta = 0.70$ ,  $P = 0.013$ ).

In multivariable linear regression analysis concerning age, sex, DR severity, and the presence of hypertension, a significant association was observed between FDa and VAD<sub>SCP</sub> ( $\beta = 0.40$ ,  $P < 0.001$ ), FDa and VLD<sub>SCP</sub> ( $\beta = 0.01$ ,  $P < 0.001$ ), and BCa and FAZ circularity ( $\beta = -1.02$ ,  $P = 0.001$ ; see Table 5).

### DISCUSSION

In the present study, retinal vessel geometry and OCTA parameters were investigated in the same eyes of patients



with DR to obtain wholistic representations of microvascular changes in arterioles, venules, and capillaries. We also investigated the associations between retinal vessel geometry parameters and OCTA metrics. DR severity was negatively associated AVR, FDA, JEa, JEv, VAD<sub>SCP</sub>, VAD<sub>DCP</sub>, VLD<sub>SCP</sub>, VLD<sub>DCP</sub>, and FAZ circularity. DR severity was positively associated with CRVE, BCa, BCv, and FAZ size. Among these parameters, we observed associations between FDA and VAD<sub>SCP</sub>, FDA and VLD<sub>SCP</sub>, and BCa and FAZ circularity after multivariable linear regression analysis. A similar analysis was performed in only one recent study,<sup>10</sup> but only a limited number of retinal vessel geometry and OCTA parameters were analyzed in a small number of patients.

Several studies have evaluated the relationship between retinal vessel geometry and DR severity but report inconsistent results. Most studies reported wider venular caliber in severe DR, consistent with our finding.<sup>8,11,12</sup> Alternatively, many studies present contradictory results regarding arteriolar width, and the AVR also varied between studies, accordingly.<sup>11</sup> Several studies indicate conflicting results regarding the association between the DR severity and the vascular fractal dimension.<sup>13</sup> In addition, the fractal dimension was calculated without distinguishing between the arteriole and the venule in many studies.

More studies report an increased vascular tortuosity in severe DR than a decrease.<sup>8,11</sup> However, other studies indicate no association between the vascular tortuosity and DR severity, consistent with our results.<sup>13</sup> Although there are few studies evaluating branching coefficient or junctional exponent deviation in DR, it has been reported that JEa decreased in severe DR, consistent with our finding.<sup>11</sup> In contrast, most studies of OCTA parameter change according to DR severity report consistent results<sup>14</sup>: lower VAD, lower VLD, decreased FAZ circularity, and enlarged FAZ area, similar to our findings.

It has been suggested that such retinal vascular geometry parameters reflect the optimal state of retinal circulation.<sup>15</sup> Therefore, hemodynamic changes associated with pathophysiological processes in DR are considered to alter the vascular geometry. Data generated in this study provide evidence to support the hypothesis that alterations in retinal vascular geometry reflect different pathophysiological changes in retinal capillaries in DR.

Our study findings showed that a decreased FDA in severe DR was associated with decreased VAD<sub>SCP</sub> and VLD<sub>SCP</sub>. Grauslund et al.<sup>16</sup> proposed that reduced blood flow may occur in response to lower metabolic demand due to the destruction of viable retinal tissue. This results in narrowing of the vessel diameter, followed by a comparative decrease in detection capacity using software, which leads to a smaller fractal dimension. The decreased SCP vascular density measurements (both VAD<sub>SCP</sub> and VLD<sub>SCP</sub>) noted in our study largely reflect retinal capillary closure. Because retinal capillary closure results in a reduction in retinal blood flow,<sup>17</sup> both decreased VAD<sub>SCP</sub> and VLD<sub>SCP</sub> can be associated with a decrease in the FDA. Interestingly, FDA was significantly associated with only the vascular density measurements of SCP, not those of DCP. Onish et al.<sup>18</sup> speculated that autoregulation of SCP blood flow may be more preserved with progressing DR compared with DCP. This suggests that the retinal vascular autoregulation impairment, which is the background for the decrease in FDA in severe DR,<sup>19</sup> causes more vascular density parameter changes in SCP than DCP. This is supported by a recent finding that the increased reti-

nal arterial blood flow due to flicker-light stimulation, which is mediated by the retinal vascular autoregulation, was more correlated with the vascular density of SCP than DCP.<sup>20</sup>

Increased BCa in severe DR was found to be associated with decreased FAZ circularity. BCa indicates the ratio of the branching daughter arteriole widths to trunk arteriole width at the bifurcation. An increased BCa suggests decreased daughter arteriole widths or an increased trunk width. However, decreased JEa was also noted in severe DR, which showed that an increased BCa resulted from decreased daughter arteriole widths. Optimal design of vessel bifurcations achieves the most efficient blood flow transport with minimum energy spent due to constant shear stress.<sup>20</sup> The endothelium plays a key role largely in the optimal design achievement of vessel bifurcations via the production of nitric oxide (NO) in response to shear stress.<sup>21</sup> Endothelial dysfunction in severe DR may result in dysfunction of NO production, followed by decreased daughter arteriole widths, which leads to an increased BCa. Similarly, endothelial dysfunction causes uneven dropout of capillaries at the FAZ border, resulting in decreased FAZ circularity. We consider that BCa changes could result from endothelial dysfunction, as indicated through FAZ circularity.

The limitations in this study stem primarily from its retrospective design. To minimize the limitations of this retrospective study, we analyzed a relatively large number of consecutive patients who met the inclusion criteria. However, the study limitations are as follows. First, associations identified in this study do not directly imply temporality or causality due to the study's retrospective and cross-sectional design. Second, refractive error, axial length, smoking status, and cardiovascular disease, which may influence retinal vascular parameters, could not be evaluated in the analysis. Furthermore, hypertension data might have affected our study results, as 42.9% of the patients had hypertension. However, there was no difference in the number of patients with hypertension among the DR severity groups, and we adjusted for the presence of hypertension in the multivariable linear regression analysis. Third, healthy individuals without DM were included in the analysis. Even in patients with no DR showed a lesser degree, but a similar trend of retinal vessel geometry parameters and OCTA metric changes as in apparent DR.<sup>22,23</sup> This suggests that patients with no DR may not be suitable controls. Future prospective and longitudinal studies that include healthy controls, and adjusting for other possible confounders, are needed to confirm our study findings.

Fourth, despite analysis of retinal vessel geometry using SIVA software being semi-automated, a degree of subjective human interaction was required, with potential intergrader and intragrader variability. Due to limitations in the SIVA software, substantial manual corrections were undertaken; however, high intergrader reliability was observed in our study. Finally, we evaluated only two layers of the vascular plexus, namely, the SCP and DCP. The recently developed high-resolution OCTA can segment additional vascular layers and visualize the pre-capillary arteriole, which is known to play an important role in retinal vascular function and autoregulation.<sup>24</sup> A further study using high-resolution OCTA could contribute to expanding our understanding of the pathophysiology in DR.

In conclusion, geometric changes in the retinal arterioles and venules in DR were found to be associated with changes in retinal capillary parameters indicated by OCTA metrics. Our findings supported the hypothesis that geometric

alterations in retinal arterioles and venules in DR may result from alterations in retinal capillaries.

### Acknowledgments

Supported by the Division of Biostatistics, Hallym Institute for Clinical Medicine, Hallym University Medical Center.

Disclosure: **J.M. Choi**, None; **S.M. Kim**, None; **Y.H. Bae**, None; **D.J. Ma**, None

### References

1. Wilkinson CP, Ferris FL 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110:1677–1682.
2. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab*. 2013;17:20–33.
3. Paul S, Ali A, Katare R. Molecular complexities underlying the vascular complications of diabetes mellitus - A comprehensive review. *J Diabetes Complications*. 2020;34:107613.
4. Hammes HP, Feng Y, Pfister F, Brownlee M. Diabetic retinopathy: targeting vasoregression. *Diabetes*. 2011;60:9–16.
5. McGrory S, Taylor AM, Kirin M, et al. Retinal microvascular network geometry and cognitive abilities in community-dwelling older people: The Lothian Birth Cohort 1936 study. *Br J Ophthalmol*. 2017;101:993–998.
6. Klein R, Lee KE, Danforth L, et al. The Relationship of Retinal Vessel Geometric Characteristics to the Incidence and Progression of Diabetic Retinopathy. *Ophthalmology*. 2018;125:1784–1792.
7. Ikram MK, Cheung CY, Lorenzi M, et al. Retinal vascular caliber as a biomarker for diabetes microvascular complications. *Diabetes Care*. 2013;36:750–759.
8. Kee AR, Wong TY, Li LJ. Retinal vascular imaging technology to monitor disease severity and complications in type 1 diabetes mellitus: A systematic review. *Microcirculation*. 2017;24:12327.
9. Tey KY, Teo K, Tan ACS, et al. Optical coherence tomography angiography in diabetic retinopathy: a review of current applications. *Eye Vis (Lond)*. 2019;6:37.
10. Tan TE, Nguyen Q, Chua J, et al. Global Assessment of Retinal Arteriolar, Venular and Capillary Microcirculations Using Fundus Photographs and Optical Coherence Tomography Angiography in Diabetic Retinopathy. *Sci Rep*. 2019;9:11751.
11. Crosby-Nwaobi R, Heng LZ, Sivaprasad S. Retinal vascular calibre, geometry and progression of diabetic retinopathy in type 2 diabetes mellitus. *Ophthalmologica*. 2012;228:84–92.
12. Cheung CY, Lamoureux E, Ikram MK, et al. Retinal vascular geometry in Asian persons with diabetes and retinopathy. *J Diabetes Sci Technol*. 2012;6:595–605.
13. Forster RB, Garcia ES, Sluiman AJ, et al. Retinal venular tortuosity and fractal dimension predict incident retinopathy in adults with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetologia*. 2021;64:1103–1112.
14. Sun Z, Yang D, Tang Z, Ng DS, Cheung CY. Optical coherence tomography angiography in diabetic retinopathy: an updated review. *Eye (Lond)*. 2021;35:149–161.
15. Zamir M. Optimality principles in arterial branching. *J Theor Biol*. 1976;62:227–251.
16. Grauslund J, Green A, Kawasaki R, Hodgson L, Sjolie AK, Wong TY. Retinal vascular fractals and microvascular and macrovascular complications in type 1 diabetes. *Ophthalmology*. 2010;117:1400–1405.
17. Fu X, Gens JS, Glazier JA, Burns SA, Gast TJ. Progression of Diabetic Capillary Occlusion: A Model. *PLoS Computational Biology*. 2016;12:e1004932.
18. Onishi AC, Nesper PL, Roberts PK, et al. Importance of Considering the Middle Capillary Plexus on OCT Angiography in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci*. 2018;59:2167–2176.
19. Broe R, Rasmussen ML, Frydkjaer-Olsen U, et al. Retinal vascular fractals predict long-term microvascular complications in type 1 diabetes mellitus: the Danish Cohort of Pediatric Diabetes. 1987 (DCPD1987). *Diabetologia*. 2014;57:2215–2221.
20. Murray CD. The Physiological Principle of Minimum Work: I. The Vascular System and the Cost of Blood Volume. *Proc Natl Acad Sci USA*. 1926;12:207–214.
21. Järvisalo MJ, Raitakari M, Toikka JO, et al. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation*. 2004;109:1750–1755.
22. Sun C, Liew G, Wang JJ, et al. Retinal vascular caliber, blood pressure, and cardiovascular risk factors in an Asian population: the Singapore Malay Eye Study. *Invest Ophthalmol Vis Sci*. 2008;49:1784–1790.
23. Dimitrova G, Chihara E, Takahashi H, Amano H, Okazaki K. Quantitative retinal optical coherence tomography angiography in patients with diabetes without diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2017;58:190–196.
24. Muraoka Y, Uji A, Ishikura M, Iida Y, Ooto S, Tsujikawa A. Segmentation of the Four-Layered Retinal Vasculature Using High-Resolution Optical Coherence Tomography Angiography Reveals the Microcirculation Unit. *Invest Ophthalmol Vis Sci*. 2018;59:5847–5853.
25. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143–149.