# Macular Vascular Fractal Dimension in the Deep Capillary Layer as an Early Indicator of Microvascular Loss for Retinopathy in Type 2 Diabetic Patients

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**Purpose.** To determine the ability of fractal dimension to detect early changes in the retinal microvascular network imaged by optical coherence tomography angiography (OCT-A) in type 2 diabetic patients.

**Methods.** Sixty-seven patients with type 2 diabetic mellitus (DM) (48 with no diabetic retinopathy [DR], 19 with minimal DR) and 40 control subjects. Macular OCT-A images of the superficial and deep retinal capillary layers in a 2.5-mm diameter concentric annular zone (excluding the foveal avascular zone) were subdivided into six annular rings and four quadrants. A custom automated algorithm was developed to quantify the complexity and density of the two retinal capillary layers by fractal analysis.

**Results.** Compared to controls, the fractal dimensional parameter ($D_{box}$) of the two retinal capillary layers in most regions was significantly lower in diabetic patients with minimal DR ($P < 0.05$). The $D_{box}$ of the diabetic patients with no DR was also decreased in most regions of the deep retinal capillary layer ($P < 0.05$), but not in the superficial retinal capillary layer ($P > 0.05$). Based on the receiver operating characteristic curve analysis, the $D_{box}$ values for the deep retinal capillary layer had the highest index to discriminate diabetic patients with and without minimal DR from controls.

**Conclusions.** Fractal dimension based on OCT-A has the potential to quantitatively characterize retinal microvascular changes in the early stage of DM. Changes in the fractal dimension in the deep retinal capillary layer could be an early indicator of microvasculature changes associated with retinopathy in type 2 diabetic patients.

Keywords: macular microvasculature, fractals, optical coherence tomography angiography, diabetic mellitus

As a main complication of diabetes mellitus (DM), diabetic retinopathy (DR) is one of the leading causes of blindness and visual impairment in the working-age population around the world.[1,2] DR is a typical type of ischemia-driven retinal disease characterized by microvascular damage to the retina. In studies of animal models, abnormal retinal microcirculation (e.g., vascular contraction[3] and apoptosis of pericytes and endothelial and microvascular cells[4]) has been widely recognized, even before the early clinical signs occur (e.g., microaneurysms). Nevertheless, no clinically quantitative parameters are available to describe the microvascular network loss in early stage DR and identify diabetic patients at risk to develop the associated retinopathy. Identifying early indicators of microvascular network loss in DM before the first clinically observable signs of DR will allow the earlier implementation of treatment and support the development of new treatment modalities.

The retinal vascular fractal dimension is a global measure derived from fractal analysis of the retinal vasculature. It quantitatively describes the complexity of the branching pattern and the density of the entire retinal vascular system. Several studies have shown that changes of fractal dimension may be an early biomarker of vascular changes and may be useful to predict the incidence and progression of retinopathy.[5–9] In those studies, the fractal dimension was assessed based on fluorescein angiography (FA) images[10] or color fundus photographs.[5,11] However, these imaging modalities are either invasive and/or they cannot image the retinal microvascular network in sufficient detail, and thus they cannot detect the subtle vascular changes in the very early stage of DM.

Optical coherence tomography angiography (OCT-A) is a novel imaging modality that noninvasively and quickly demonstrates the retinal microvasculature with high resolution. Moreover, this technique provides in-depth information, allowing the visualization of the retinal microvascular network in different retinal layers.[12–14] Recently, studies using OCT-A have revealed abnormalities of fractal dimensions in patients with DR[15–17]; however, the results remain controversial. Importantly, it is noteworthy that the OCT-A studies did not evaluate changes of fractal dimension in diabetic patients with no DR. Evidence from histopathologic studies has shown that changes in the retinal capillaries precede clinical retinal signs.[18,19] Therefore, we sought to further determine the utility of OCT-A-derived fractal dimensions in detecting preclinical

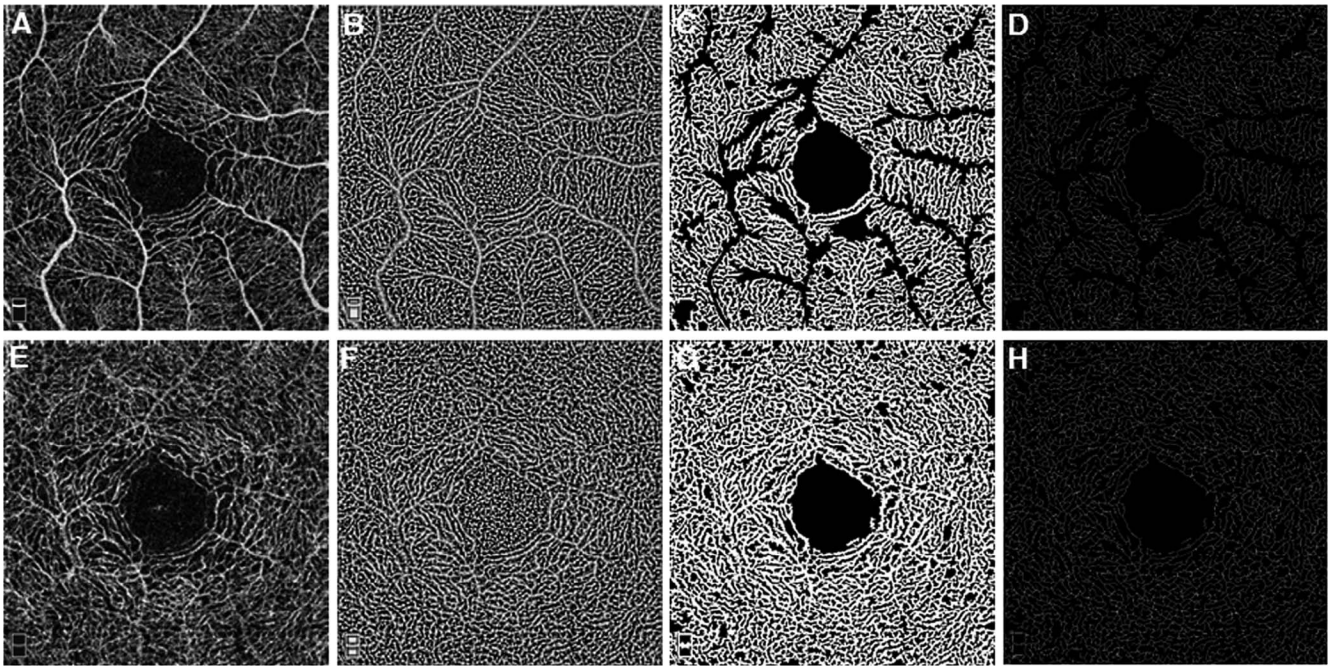


FIGURE 1. Representative OCT-A and postprocessed images for a control eye illustrating the automated analysis algorithm. (**A**, **E**) Original OCT-A images of the superficial (**A**) and deep (**E**) retinal capillary layers. (**B**, **F**) Contrast-enhanced images of the superficial (**B**) and deep (**F**) retinal capillary layers. (**C**, **G**) Binary images of the microvascular network after removing the large vessels with a width $\geq$25 μm and background noise in the superficial (**C**) and deep (**G**) retinal capillary layers. (**D**, **H**) Skeletonized images of the superficial (**D**) and deep (**H**) retinal capillary layers. The skeletonized images were used for fractal analysis.

lesions of retinal capillaries in DM and predicting the development of retinopathy. Thus, the aim of this study was to use fractal analysis of OCT-A images to characterize the macular microvascular network in type 2 diabetic patients with either no or minimal clinical DR. We then determined the ability of fractal dimension to detect early changes in the retinal microvascular network of these patients.

## METHODS

### Patient Data Collection

In this retrospective study, all the type 2 diabetic patients were from the endocrine department of the Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University and diagnosed by an endocrine specialist (CW). Approval for data collection and analysis was obtained from the ethics committee of the Eye Hospital of Wenzhou Medical University and adhered to the tenets set forth in the Declaration of Helsinki.

Demographic information collected from the patients included age, sex, body mass index (BMI), duration of DM, blood glucose (BG), HbA1c level, and systolic/diastolic blood pressure (SBP/DBP). All the diabetic patients had an extensive ophthalmologic examination, including slit-lamp biomicroscopy, intraocular pressure (IOP) measurement, refraction and best corrected visual acuity (BCVA) test, axial length (AL) measurement, and ophthalmoscopy. Inclusion criteria included the diagnosis of type 2 DM with either no or minimal DR, as determined by two retinal specialists (FC and RZ). Minimal DR was defined in accordance with the second stage of the International Clinical Diabetic Retinopathy Disease Severity Scale.[20] Thus it was characterized by the presence of at least one microaneurysm or hemorrhage in the central retina but no other diabetic lesions. Exclusion criteria were as follows: refractive errors over +2.00 diopters (D) or under −6.00 D of spherical equivalent (SE) or −1.50 D of astigmatism, visual acuity less than 20/25, significant media opacities, IOP $>$ 21 mm Hg or previous diagnosis of glaucoma, uveitis, or retinal disease, as well as insufficient ability to fixate in the OCT-A examination. Age- and sex-matched control subjects were recruited from the students and workers at the Eye Hospital of Wenzhou Medical University. The control individuals underwent the same tests as those used to evaluate the diabetic patients, except for the analysis of BG and HbA1c level.

### Image Acquisition Protocol

After the above screening tests, all the enrolled subjects were imaged by an OCT system (Optovue RTVue XR Avanti; Optovue, Inc., Fremont, CA, USA) to obtain the OCT-A images, as previously described.[14] The scan speed was 70,000 A-scans per second and the scan area, centered on the fovea, was $3 \times 3$ mm$^2$, obtained by orthogonal registration and merging of two consecutive scans. The size of the exported OCT images was $304 \times 304$ pixels. A good set of scans with a signal strength index of $>$40 for each eye was selected for further analysis. The superficial and deep retinal capillary plexuses were detected and separated automatically by the OCT instrument (Figs. 1A, 1E). The superficial retinal capillary layer extended from 3 μm below the internal limiting membrane to 15 μm below the inner plexiform layer (IPL). The deep retinal capillary layer extended from 15 to 70 μm below the IPL.

### Quantitative Analysis of OCT Angiography Images Based on Fractal Analysis

To quantify the complexity and density of the retinal microvascular network in the OCT-A images, we used a custom automated fractal analysis algorithm that included a correction of the image magnification based on the AL.[21–23] In brief, the

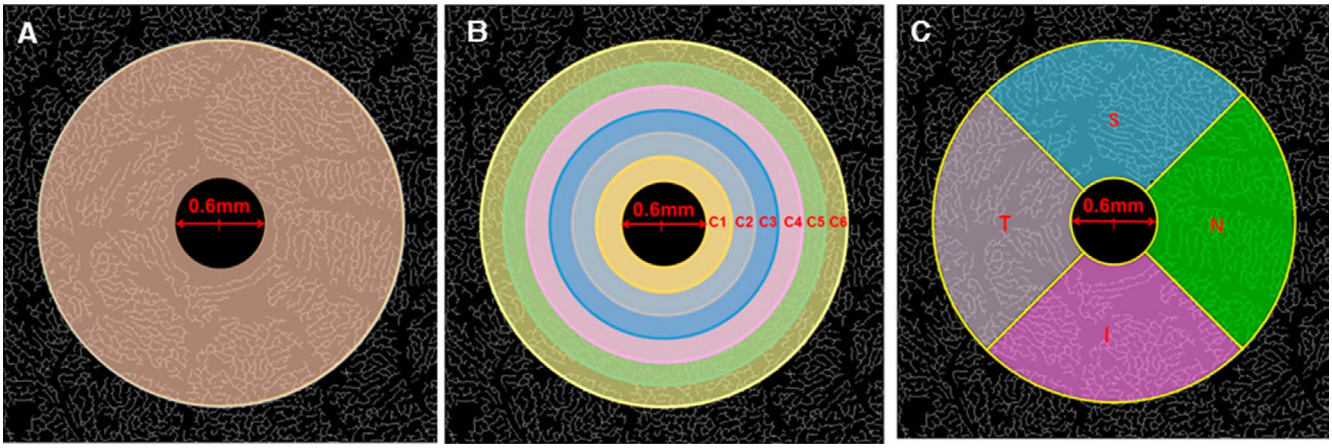


FIGURE 2. Diagrammatic sketch illustrating the fractal analysis on the skeletonized OCT-A image. Three forms of analyzed regions were used to describe the general and local fractal dimensions after excluding the FAZ from each region. (**A**) Fractal analysis was performed on the total annular zone with a diameter of 2.50 mm after excluding the FAZ (diameter = 0.60 mm). (**B**) Fractal analysis was performed in six annular zones, C1 (diameter = 0.92 mm), C2 (diameter = 1.23 mm), C3 (diameter = 1.55 mm), C4 (diameter = 1.87 mm), C5 (diameter = 2.18 mm), and C6 (diameter = 2.50 mm). (**C**) Fractal analysis was performed in the parafoveal four quadrant sectors, S, T, I, and N, of a circular zone with a diameter of 2.50 mm after excluding the FAZ.

OCT-A images in PNG format were exported from the OCT-A device to the custom automated algorithm software. The grayscale of each two-dimensional OCT-A image was first extended by bicubic interpolation to 1024 × 1024 pixels to enhance the image details. Then, the image was segmented to obtain the microvascular network. First, the boundary foveal avascular zone (FAZ) was detected by using a two-way combined method consisting of a canny edge detector algorithm and a level set algorithm (Figs. 1B, 1F for the superficial and deep retinal capillary layers, respectively). The area within the FAZ having a circle of fixed radius (diameter = 0.6 mm) was then determined to establish the baseline signal-to-noise ratio for the global thresholding.

This image was then separately processed to generate two binary images: the first one contained only the large blood vessels and was generated by using global thresholding, a local gray-level change enhancement algorithm called "gray-voting," Gabor filtering, and adaptive thresholding. The other binary image contained the large and small vessels and was created by using global thresholding, gray-voting algorithm, and adaptive thresholding. Lastly, the two resulting binary vessel maps were subtracted to obtain the binary image containing only the small vessels (Figs. 1C, 1G for the superficial and deep retinal capillary layers, respectively). Based on the final binary image, a skeletonized image was created by detecting the central axis of the binary, white-pixelated vasculature, and remaining one pixel along the central axis (Figs. 1D, 1H for the superficial and deep retinal capillary layers, respectively).

After the image processing, fractal analysis was performed on the skeletonized images of both the superficial and deep retinal capillary layers. The quantitative measured parameter of fractal dimension, $D_{box}$, was obtained using the fractal analysis toolbox from Benoit (TruSoft Benoit Fractal Analysis Toolbox, TruSoft International, Inc., St. Petersburg, FL, USA). Three forms of the analyzed regions were used to describe the general and local complexity of the microvasculature (Fig. 2). First, the fractal dimension was calculated for the 2.5-mm diameter total annular zone after excluding the FAZ (diameter = 0.6 mm, Fig. 2A). Fractal dimensions were then automatically calculated in six concentric isometric annular rings after excluding the FAZ (Figs. 2B, C1–C6). Fractal dimensions were also automatically calculated in the parafoveal quadrant sectors (i.e., superior [S], temporal [T], inferior [I], and nasal [N] sectors of the 2.5-mm diameter circular zone after excluding the FAZ [Fig. 2C]). All the above methods were implemented using MATLAB v7.10 (Mathworks, Inc., Natick, MA, USA).

### Data Analysis and Statistical Methods

All data were expressed as the means ± standard deviations and were analyzed with SPSS software (version 22.0; SPSS, Inc., Chicago, IL, USA). Refraction data were converted to SEs, calculated as the spherical dioptric power plus one-half of the cylindrical dioptric power. One-way analysis of variance (ANOVA) was used to test for differences among the control subjects, diabetic patients with no DR, and diabetic patients with minimal DR, and post hoc tests were used between group pairs. The differences between sexes within each of the three groups were determined by the $\chi^2$ test. The receiver operating characteristic (ROC) curve analysis was calculated to evaluate the ability of the OCT-A-based vessel fractal analysis to determine the microvascular network impairment in the early stage of DR. Larger areas under the ROC curve (AUC) indicated higher diagnostic value. A value of $P < 0.05$ was considered statistically significant.

## RESULTS

### Patient Characteristics

A total of 67 consecutive patients with type 2 DM (48 with no DR; 19 with minimal DR) and 40 control subjects were included in the study. There were no significant differences among the controls, DM patients with no DR, and DM patients with minimal DR in age, sex, BMI, SE, BCVA, AL, IOP, duration of DM, BG, HbA1c level, and SBP/DBP (Table 1).

### Intergroup Differences Among the Three Groups: Fractal Analysis

Fundus photographs and raw OCT-A images of the superficial and deep retinal capillary layers in control eyes (Figs. 3A, 3D, 3G) and in eyes of diabetic patients with no DR (Figs. 3B, 3E, 3H) and with minimal DR (Figs. 3C, 3F, 3I) were acquired. In eyes with minimal DR, the presence of an abnormal retinal vasculature was evident as a decrease in capillary complexity

**Table 1.** Characteristics of Type 2 Diabetic Patients With No and Minimal DR and Controls

| | Control, $n = 40$ | No DR, $n = 48$ | Minimal DR, $n = 19$ | *P* Value |
|---|---|---|---|---|
| Eyes | 40 | 74 | 29 | – |
| Age, y | 52.58 ± 6.97 | 53.92 ± 10.11 | 58.16 ± 12.12 | 0.109* |
| Sex, M/F | 16/24 | 27/21 | 10/9 | 0.302† |
| BMI | 22.80 ± 2.94 | 24.00 ± 3.09 | 23.95 ± 3.41 | 0.298* |
| SE | 0.35 ± 1.30 | 0.03 ± 1.33 | -0.00 ± 1.50 | 0.566* |
| BCVA, LogMAR | 0.02 ± 0.03 | 0.02 ± 0.06 | 0.04 ± 0.06 | 0.097* |
| AL, mm | 22.91 ± 1.61 | 23.19 ± 0.8 | 22.95 ± 0.77 | 0.386* |
| IOP, mm Hg | 13.20 ± 2.85 | 14.43 ± 3.22 | 15.59 ± 3.73 | 0.090* |
| SBP, mm Hg | 128.27 ± 14.12 | 129.77 ± 18.77 | 136.94 ± 19.58 | 0.233* |
| DBP, mm Hg | 79.00 ± 8.78 | 79.79 ± 12.40 | 78.74 ± 10.47 | 0.924* |
| MAP, mm Hg | 122.09 ± 12.68 | 123.05 ± 17.21 | 124.39 ± 15.68 | 0.893* |
| Duration, y | NA | 5.28 ± 5.12 | 6.81 ± 4.14 | 0.261‡ |
| BG, mmol/L | – | 8.22 ± 5.15 | 8.25 ± 2.73 | 0.983‡ |
| HbA1c, % | – | 8.20 ± 2.26 | 8.81 ± 1.89 | 0.340‡ |

Values are means ± SD for all subjects in each group. Control, control eyes; No DR, diabetic patients with no DR; minimal DR, diabetic patients with minimal DR; –, not performed; NA, not applicable, M, male; F, female; MAP, mean arterial pressure; HbA1c (%), glycosylated hemoglobin.

* ANOVA.

† $\chi^2$ test.

‡ *t*-test.

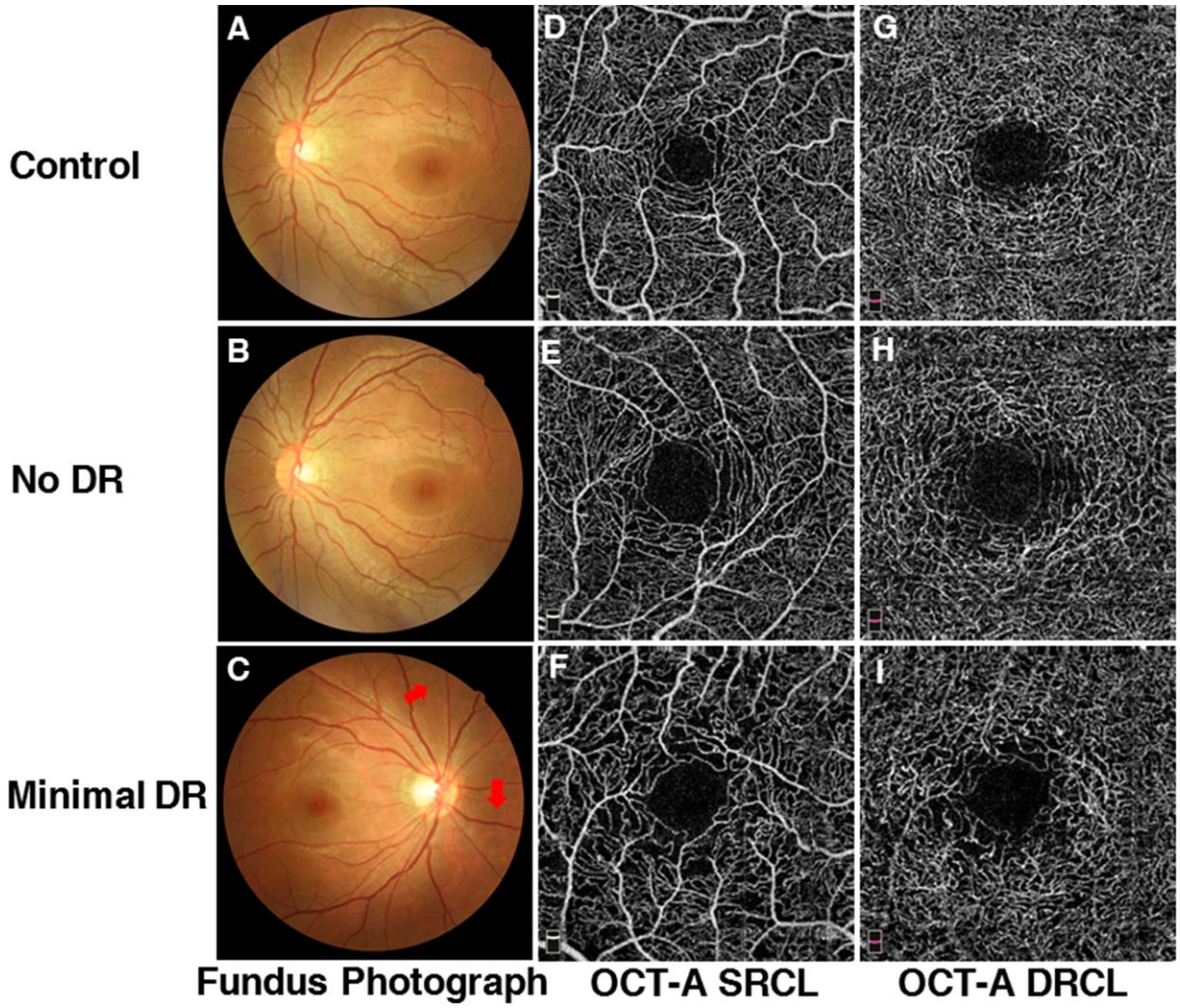


**Figure 3.** Representative fundus photographs and OCT-A images in the superficial and deep capillary layers of a control eye, an eye with no DR, and an eye with minimal DR. The images were acquired in 3 × 3-mm areas around the fovea. (**A–C**) Fundus photograph of a control, an eye with no DR, and an eye with minimal DR, respectively. The *red arrow* marks a microaneurysm in the retina of a patient with minimal DR. En face OCT-As of control, no DR, minimal DR subjects demonstrate the visualization of the superficial retinal capillary layer (**D–F**) and the deep retinal capillary layer (**G–I**). SRCL, superficial retinal capillary layer; DRCL, deep retinal capillary layer.

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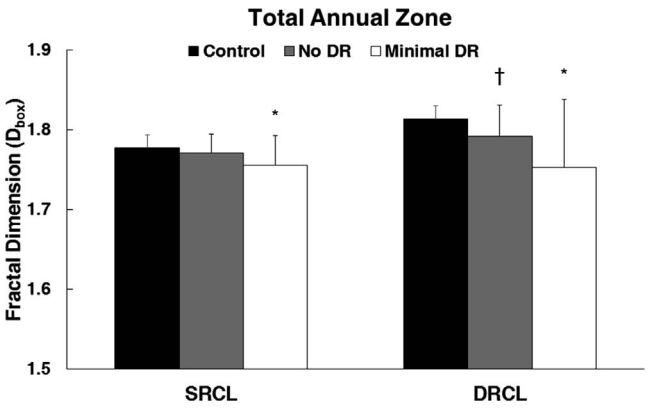


FIGURE 4. Comparison of the microvascular fractal dimensions in the total annular zone on OCT-A images in the superficial and deep retinal capillary layers. For the superficial retinal capillary layer, $D_{box}$ in minimal DR group was lower than that in control group (*$P = 0.001$). For the deep retinal capillary layer, the $D_{box}$ in the no DR group and the minimal DR group were both lower than in control group (*$P = 0.025$ and †$P < 0.001$, respectively). SRCL, superficial retinal capillary layer; DRCL, deep retinal capillary layer.

and density, especially in the deep retinal vascular layer (Fig. 3I).

$D_{box}$ values in the total annular zone were significantly lower in the superficial ($P = 0.001$) and deep ($P < 0.001$) capillary layers of the minimal DR patients compared with the control group (Fig. 4; Table 2). While the $D_{box}$ value of total annular zone superficial retinal capillary layer in diabetic patients with no DR was not different from the controls ($P = 0.248$; Fig. 4; Table 2), in the deep retinal vascular layer it was smaller than in the control eyes ($P = 0.025$; Fig. 4; Table 2). In addition, the $D_{box}$ value in the total annular zone was lower in the diabetic patients with minimal DR than in those with no DR, both in the superficial ($P = 0.004$) and deep ($P < 0.001$) retinal vascular layers (Table 2).

The mean $D_{box}$ values of the superficial and deep retinal capillary layers in the six annular zones (C1–C6, Fig. 5; Table 3) and the four quadrant sectors (S, T, I, N, Fig. 6; Table 3) around the parafovea were compared among the three groups. For the superficial retinal capillary layer, eyes with minimal DR had lower $D_{box}$ values in C4–C6, as well as in the S sector, compared to control eyes ($P < 0.05$; Figs. 5A, 6A; Table 3). However, in eyes with no DR, the $D_{box}$ values in all annular zones (Fig. 5A; Table 3) and in all quadrant sectors (Fig. 6A; Table 3) were not different from those in the control eyes ($P > 0.05$).

For the deep retinal capillary layer in eyes with minimal DR, the $D_{box}$ values for microvascular complexity and density in all annular zones (Fig. 5B; Table 3) and in all quadrant sectors (Fig. 6B; Table 3) were significantly lower than in control eyes ($P < 0.05$). Diabetic patients with no clinical DR had significantly lower $D_{box}$ values for microvascular complexity and density in all annular zones except C1 ($P = 0.805$) and C2 ($P = 0.241$) (Fig. 5B; Table 3) and in all quadrants except the I ($P = 0.078$) and N sectors ($P = 0.097$) (Fig. 6B; Table 3) compared to the control eyes ($P < 0.05$). The $D_{box}$ values for all annular zones and quadrant sectors were lower in diabetic patients with minimal DR compared to those with no DR ($P < 0.05$; Table 3).

## ROC Curve Analysis

We performed the ROC curve analysis to determine the ability of fractal dimensional parameters to detect the early microvascular changes in the diabetic patients with either no or minimal DR. Overall, the AUCs of the $D_{box}$ values in the deep retinal capillary layer were generally larger than those in the superficial retinal capillary layer for diabetic patients with either no or minimal DR. For the total annular zones, the $D_{box}$ value in the deep retinal capillary layer had a high ability to discriminate between eyes with minimal DR and control eyes (AUC = 0.900, sensitivity = 67.5%, specificity = 100%, $P < 0.001$; Fig. 7D; Table 4). Additionally, the $D_{box}$ value in the deep retinal capillary layer had a significant but moderate ability to discriminate between diabetic patients with no DR and control eyes (AUC = 0.735, sensitivity = 67.5%, specificity = 73.0%, $P < 0.001$; Fig. 7A; Table 4).

Among the six annular zones of the deep retinal capillary layer, the $D_{box}$ value in C4 had the highest index to discriminate diabetic patients with minimal DR from control subjects (AUC = 0.866, sensitivity = 90.0%, specificity = 67.9%, $P < 0.001$; Fig. 7E; Table 4). The $D_{box}$ value in C3 had the highest index to discriminate diabetic patients with no DR from control subjects (AUC = 0.740, sensitivity = 55.0%, specificity = 86.5%, $P < 0.001$; Fig. 7B; Table 4). In the four quadrants, the $D_{box}$ value in the N sector had the highest index to discriminate diabetic patients with minimal DR from control subjects (AUC = 0.861, sensitivity = 77.5%, specificity = 82.1%, $P < 0.001$; Fig. 7F; Table 4). The $D_{box}$ value in the T sector had the highest index to discriminate diabetic patients with no DR from control subjects (AUC = 0.695, sensitivity = 77.5%, specificity = 59.5%, $P = 0.001$; Fig. 7C; Table 4).

## DISCUSSION

The retinal microvasculature is an early and prevalent target of diabetic damage. The change in the retinal vascular network of diabetic patients is viewed as an indicator of retinopathy onset and development.[8,9] Fractal analysis can provide more insights into the development of retinal vascular diseases[24,25] compared to some other geometric measures, such as the caliber of the retinal vessels.[8,26,27] Previous studies using some non-OCT-A technologies, including FA and fundus photographs, have attempted to use fractal dimension to evaluate the early changes of retinal vasculature in DM with no or early-stage DR; however, the findings were conflicting or nonsignificant (Table 5). The use of FA and fundus photos is a common limitation of these previous studies, which cannot qualitatively detect the

TABLE 2. $D_{box}$ in the Total Annular Zone of the Superficial and Deep Retinal Capillary Layers

| | Control | No DR | Minimal DR | *P** | *P*† | *P*‡ |
|---|---|---|---|---|---|---|
| SRCL | 1.777 ± 0.016 | 1.771 ± 0.023 | 1.755 ± 0.038 | 0.248 | 0.001 | 0.004 |
| DRCL | 1.813 ± 0.017 | 1.791 ± 0.040 | 1.753 ± 0.085 | 0.025 | <0.001 | <0.001 |

Control, control eyes; no DR, diabetic patients with no DR; minimal DR, diabetic patients with minimal DR; SRCL, superficial retinal capillary layer; DRCL, deep retinal capillary layer.

* *P* value for the comparison between the no DR and control groups.

† *P* value for the comparison between minimal DR and control groups.

‡ *P* value for the comparison between no DR and minimal DR groups.

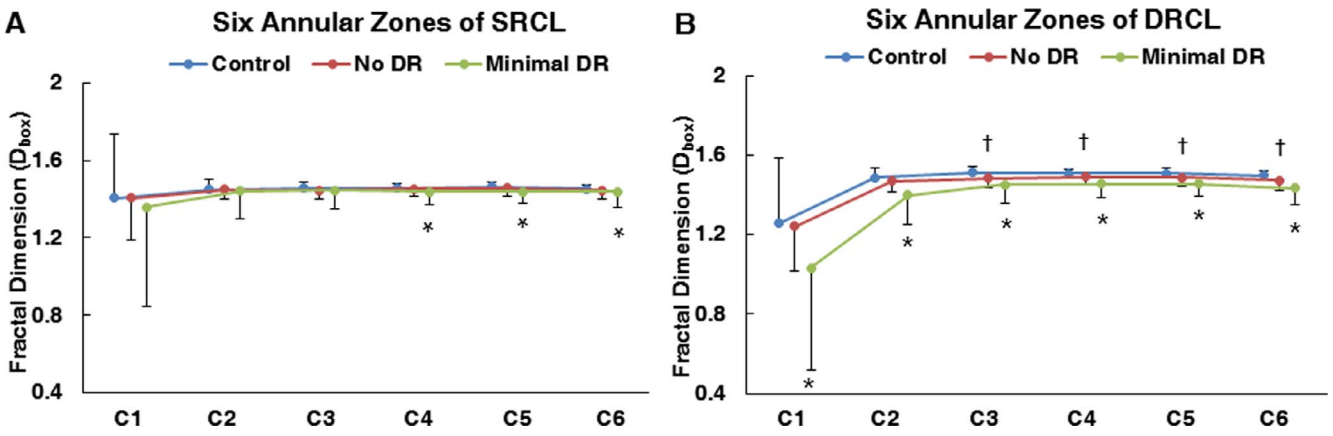


FIGURE 5. Comparisons of the microvascular Dbox on OCT-A images in the six annular zones of the superficial retinal capillary layer (SRCL) (**A**) and deep retinal capillary layer (DRCL) (**B**). *$P < 0.05$, the $D_{box}$ in the minimal DR group were lower than that in control group; †$P < 0.05$, the $D_{box}$ in the no DR group were lower than that in control group.

early subtle changes in retinal vasculature at the capillary level. OCT-A enables noninvasive imaging of the retinal capillaries. It is especially useful for the in-depth analysis of retinal vessels in multiple layers that were previously invisible on fundus images. To the best of our knowledge, the present use of fractal analysis of OCT-A images is the first to characterize changes of the microvasculature complexity in type 2 diabetic patients with no clinical retinopathy. Our study showed that fractal dimensions in the deep retinal capillary layer were significantly lower in type 2 diabetic patients with no DR compared with the nondiabetic control subjects. This suggests that the complexity of the vasculature decreases in patients with type 2 DM before the clinical signs occur. Our study strongly suggested that fractal analysis from OCT-A images can identify preclinical lesions of the retinal capillaries in DM before the manifestation of clinically apparent retinopathy.

Patients with type 2 DM with minimal DR in our study had decreased $D_{box}$ values in the total annular zone in both the superficial and deep retinal capillary layers compared with healthy subjects. Our results are in agreement with previous studies using same or different OCT-A algorithm.[9,10,17] Interestingly, diabetic patients with no clinical retinopathy had decreased $D_{box}$ values of the total annular zone only in the deep retinal capillary layer. This indicates that the early changes of retinal capillaries associated with DR may first occur in the deep retinal capillary layer. In addition, we found there was a dramatically greater decrease in fractal dimensions in eyes with minimal DR compared to those with no DR, both in deep and superficial layers. This indicates that the degree of decrease in microvascular complexity correlates with and predicts the development of DR.

TABLE 3. $D_{box}$ in the Six Annular Zones and Four Quadrant Sectors in the Superficial and Deep Retinal Capillary Layers

| Layers | Regions | Control | No DR | Minimal DR | *P** | *P*† | *P*‡ |
|---|---|---|---|---|---|---|---|
| SRCL | C1 | 1.408 ± 0.096 | 1.408 ± 0.077 | 1.360 ± 0.192 | 0.995 | 0.088 | 0.059 |
| | C2 | 1.451 ± 0.042 | 1.451 ± 0.045 | 1.441 ± 0.059 | 0.997 | 0.374 | 0.324 |
| | C3 | 1.458 ± 0.033 | 1.445 ± 0.032 | 1.446 ± 0.038 | 0.056 | 0.146 | 0.920 |
| | C4 | 1.458 ± 0.023 | 1.452 ± 0.027 | 1.438 ± 0.039 | 0.327 | 0.005 | 0.023 |
| | C5 | 1.463 ± 0.027 | 1.459 ± 0.025 | 1.441 ± 0.034 | 0.391 | 0.001 | 0.003 |
| | C6 | 1.452 ± 0.019 | 1.447 ± 0.026 | 1.437 ± 0.034 | 0.322 | 0.023 | 0.098 |
| | S | 1.715 ± 0.028 | 1.705 ± 0.037 | 1.694 ± 0.032 | 0.111 | 0.010 | 0.144 |
| | T | 1.721 ± 0.027 | 1.710 ± 0.032 | 1.709 ± 0.040 | 0.092 | 0.132 | 0.872 |
| | I | 1.714 ± 0.031 | 1.717 ± 0.028 | 1.702 ± 0.043 | 0.681 | 0.136 | 0.044 |
| | N | 1.717 ± 0.027 | 1.710 ± 0.031 | 1.704 ± 0.049 | 0.313 | 0.107 | 0.372 |
| DRCL | C1 | 1.257 ± 0.331 | 1.241 ± 0.221 | 1.033 ± 0.512 | 0.805 | 0.006 | 0.005 |
| | C2 | 1.487 ± 0.048 | 1.469 ± 0.054 | 1.398 ± 0.143 | 0.241 | <0.001 | <0.001 |
| | C3 | 1.511 ± 0.030 | 1.484 ± 0.046 | 1.454 ± 0.099 | 0.017 | <0.001 | 0.019 |
| | C4 | 1.510 ± 0.020 | 1.490 ± 0.040 | 1.455 ± 0.068 | 0.020 | <0.001 | <0.001 |
| | C5 | 1.508 ± 0.025 | 1.488 ± 0.044 | 1.458 ± 0.065 | 0.020 | <0.001 | 0.003 |
| | C6 | 1.501 ± 0.023 | 1.474 ± 0.050 | 1.435 ± 0.083 | 0.011 | <0.001 | 0.001 |
| | S | 1.760 ± 0.025 | 1.740 ± 0.041 | 1.703 ± 0.076 | 0.034 | <0.001 | <0.001 |
| | T | 1.759 ± 0.029 | 1.730 ± 0.049 | 1.701 ± 0.088 | 0.009 | <0.001 | 0.020 |
| | I | 1.760 ± 0.023 | 1.740 ± 0.046 | 1.695 ± 0.102 | 0.078 | <0.001 | 0.001 |
| | N | 1.750 ± 0.024 | 1.733 ± 0.044 | 1.678 ± 0.085 | 0.097 | <0.001 | <0.001 |

Control, control eyes; no DR, diabetic patients with no DR; minimal DR, diabetic patients with minimal DR; SRCL, superficial retinal capillary layer; DRCL, deep retinal capillary layer; C1∼C6, six annular zones.

* *P* value for the comparison between the no DR and control groups.

† *P* value for the comparison between minimal DR and control groups.

‡ *P* value for the comparison between no DR and minimal DR groups.

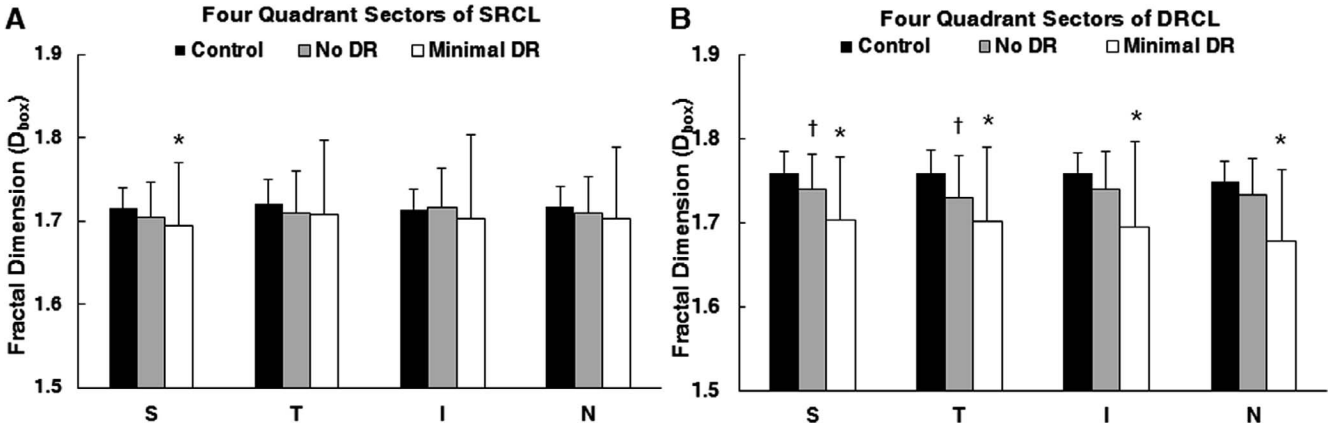


FIGURE 6. Comparisons of the microvascular Dbox on OCT-A images in the four quadrant sectors of the superficial retinal capillary layer (SRCL) (**A**) and deep retinal capillary layer (DRCL) (**B**). $^*P < 0.05$, the $D_{box}$ in the minimal DR group were lower than that in control group; $\dagger P < 0.05$, the $D_{box}$ in the no DR group were lower than in the control group.

We also analyzed the fractal dimensions in six annular zones and four quadrant sectors of the superficial and deep retinal capillary layers. Although differences in fractal dimensions in all six annular zones and four quadrant sectors of the deep retinal capillary layer were present between normal subjects and patients with minimal DR, there were significant decreases of fractal dimensions in patients with minimal DR in the outer zones (C4∼C6) and superior sector of the superficial layer. Similarly, there were significant decreases of fractal dimensions in the outer zones (C3∼C6) and in the temporal and superior sectors of the deep retinal capillary layer in patients with no DR compared to the normal subjects. This suggests that the lower fractal dimensions of the deep capillary layer found in diabetic patients before the clinical signs of retinopathy appear may be correlated with the occurrence and development of the retinopathy. Further, it suggests that retinal capillaries are affected by isolated local changes rather than diffuse alterations in very early-stage retinopathy.

The more significant decreases of fractal dimensions in the deep retinal capillary layer may be attributed to the different anatomic structures of the deep and superficial layers. The density of the smaller vessels in the deep retinal capillary layer is greater than in the superficial layer.[12,28] To compensate for the lower blood flow and the resulting hypoxia and ischemia in macula at the early stage of DM, we speculate that capillary vasoconstriction in the deep retinal capillary layer occurs earlier or more frequently than that in the superficial layer. Evidence from previous studies supports our hypothesis,

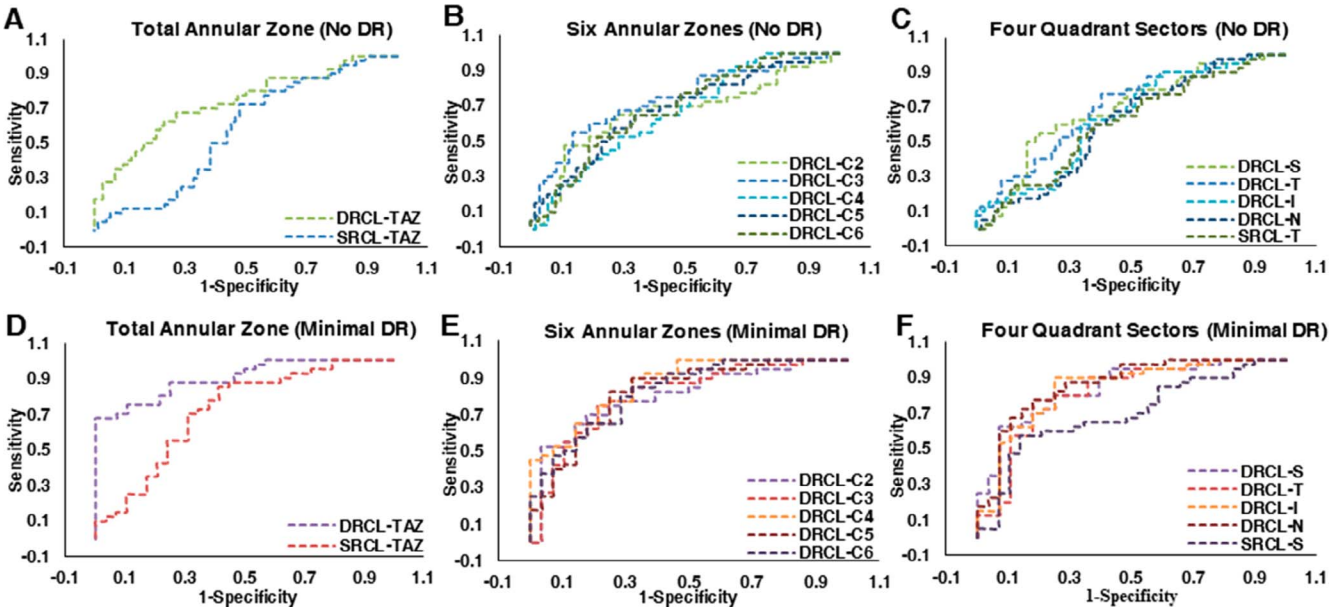


FIGURE 7. ROC curve analysis of the retinal microvascular $D_{box}$ in the total annular zone, the different annular zones and quadrant sectors. (**A**, **D**) AUCs of the total annular zones in the superficial and deep retinal capillary layers of eyes with no DR (**A**) and minimal DR (**D**). The highest AUCs, 0.735 and 0.900, were in the deep retinal capillary layers of eyes with no and minimal DR, respectively. (**B**, **E**) The five largest AUCs of the six annular zones in the superficial and deep retinal layers of the eyes with no DR (**B**) and with minimal DR (**E**). The highest AUCs, 0.740 and 0.866, were in the C3 and C4 annular zones of the deep retinal capillary layers of eyes with no DR and minimal DR, respectively. (**C**, **F**) The five largest AUCs in the four quadrant zones in the superficial and deep retinal capillary layers with no DR (**C**) and with minimal DR (**F**). The highest AUCs, 0.695 and 0.861, were in the temporal and nasal sectors of the deep retinal capillary layers of eyes with no DR and with minimal DR, respectively. TAZ, total annular zone; SRCL, superficial retinal capillary layer; DRCL, deep retinal capillary layer.

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**Table 4.** ROC Curve Analysis of $D_{box}$ in the Deep Retinal Capillary Layer of Type 2 Diabetic Patients With No and Minimal DR

| | No DR | | | | Minimal DR | | | |
|---|---|---|---|---|---|---|---|---|
| **Regions** | **AUC** | **Cutoff** | **Sen, %** | **Spe, %** | **AUC** | **Cutoff** | **Sen, %** | **Spe, %** |
| TAZ | 0.735 | 1.810 | 67.5 | 73.0 | 0.900 | 1.809 | 67.5 | 100.0 |
| C1 | 0.573 | 1.287 | 67.5 | 50.0 | 0.664 | 1.113 | 90.0 | 39.3 |
| C2 | 0.666 | 1.500 | 62.5 | 74.3 | 0.804 | 1.491 | 70.0 | 82.1 |
| C3 | 0.740 | 1.516 | 55.0 | 86.5 | 0.808 | 1.499 | 75.0 | 78.6 |
| C4 | 0.663 | 1.489 | 90.0 | 36.5 | 0.866 | 1.488 | 90.0 | 67.9 |
| C5 | 0.681 | 1.504 | 65.0 | 66.2 | 0.821 | 1.486 | 90.0 | 67.9 |
| C6 | 0.689 | 1.490 | 65.0 | 66.2 | 0.829 | 1.479 | 85.0 | 67.9 |
| S | 0.682 | 1.765 | 55.0 | 79.7 | 0.842 | 1.756 | 62.5 | 92.9 |
| T | 0.695 | 1.743 | 77.5 | 59.5 | 0.817 | 1.743 | 77.5 | 82.1 |
| I | 0.635 | 1.742 | 87.5 | 41.9 | 0.840 | 1.740 | 90.0 | 75.0 |
| N | 0.607 | 1.740 | 75.0 | 50.0 | 0.861 | 1.735 | 77.5 | 82.1 |

No DR, diabetic patiNo DR, diabetic patients with no DR; minimal DR, diabetic patients with minimal DR; TAZ, total annular zones; C1∼C6, six annular zones; Sen, sensitivity; Spe, specificity.

which suggests that the deep capillary layer is more severely affected in DM. For instance, in diabetic mice the vessel density was decreased in the deep vascular network but not in the superficial network.[28] Some previous histologic findings also revealed that vascular abnormalities were more pronounced in the deep retinal capillarylayer.[29,30]

As expected, the ROC curve analysis revealed that fractal dimensions in the deep retinal capillary layer had higher discriminating powers than the superficial retinal capillary layer to detect the earlier microvascular network loss. Because our study did not include patients with moderate or severe retinopathy or longitudinal follow-up of the patients, we

**Table 5.** Summary of Previous Studies on Fractal Dimensional Changes of the Retinal Vasculature in Diabetic Patients

| Authors | Year | Type | Groups | Vessel | Method | Alterations |
|---|---|---|---|---|---|---|
| Current study | 2017 | T2DM | No and minimal DR | Microvessels | SD-OCT-A | FD decreased in the diabetic eyes with no retinopathy compared to the normal subjects |
| Ting et al.[15] | 2017 | T2DM | No and minimal DR | Microvessels | SS-OCT-A | FD increased in the DR compared with the diabetic patients with no DR; FD was positively correlated with increasing DR severity levels |
| Zahid et al.[16] | 2016 | DM | DR | Microvessels | SD-OCT-A | FD was significantly reduced in the superficial and deep capillary plexuses in eyes with DR |
| Kim et al.[17] | 2016 | DM | DR | Microvessels | SD-OCT-A | Decreasing FD was associated with worsening DR |
| Tălu et al.[25] | 2015 | DM | NPDR | Large vessels | Fundus photographs | FD increased in mild NPDR, but decreased in moderate and more serious NPDR |
| Broe et al.[9] | 2014 | T1DM | PDR | Large vessels | Retinal photographs | Lower FD was associated with the diabetic microvascular complications |
| Cheung et al.[31] | 2012 | DM | No DR and NPDR | Large vessels | Retinal photographs | No significant changes were found in FD |
| Yau et al.[7] | 2010 | DM | DR | Large vessels | Fundus photographs | FD increased in eyes with or without overt retinopathy, but no significant changes were found in mild, moderate to severe DR |
| Grauslund et al.[11] | 2010 | T1DM | PDR | Large vessels | Retinal photographs | Lower FD was associated with PDR, neuropathy and nephropathy |
| Cheung et al.[5] | 2009 | T1DM | DR | Large vessels | Seven-field stereoscopic retinal photographs | FD increased in early diabetic microvascular damage |
| Kunicki et al.[24] | 2009 | DM | NPDR | Large vessels | Digital retinal images | No significant changes were found in FD |
| Lim et al.[6] | 2009 | T1DM | Mild NPDR | Large vessels | Seven-field stereoscopic retinal photographs | FD was not associated with incident of early DR |
| Avakian et al.[10] | 2002 | DM | NPDR | Whole vasculature | Fundus FA | FD decreased in the macula, but no changes were found in the paramacular region |
| Daxer et al.[32] | 1993 | DM | PDR | Large vessels | Low angle fundus photographs | Higher FD may have indicated proliferative changes |

T2DM, type 2 DM; T1DM, type 1 DM; NPDR, nonproliferative DR; PDR, proliferative DR; SD-OCT-A, spectral domain OCT-A; FD, fractal dimension; SS-OCT-A, swept-source OCT-A.

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cannot determine if the retinal microvascular fractal dimensions, especially in the deep retinal capillary layer, could predict the development of DR. However, our results nonetheless suggest that OCT-A with fractal analysis of the multiple retinal capillary layers can be used to identify preclinical lesions of retinal capillaries related to retinopathy in diabetic patients and may constitute an early indicator to predict DR development. Identifying the microvascular changes in diabetic patients before the occurrence of the apparent retinopathy signs may help clinicians to apply earlier implementation of treatment and may potentially be useful in predicting the progression of the microvascular complications related to DM, such as DR.

The current study used fractal analysis based on split-spectrum, amplitude-decorrelation angiography algorithm (SSDAA) from SD-OCT-A to evaluate the changes of microvascular complexity in type 2 DM. Our approach was different from that of Kim et al.,[17] in which fractal dimensions were evaluated in the whole inner retinal layer in DR patients. They used SD-OCT-A with intensity-based optical microangiography algorithm and reported fractal dimension in the whole inner retinal layer, extending from the inner limiting membrane to 110 μm above the retinal pigment epithelium. In our study, the algorithm from OCT-A allowed visualization of two distinct retinal capillary plexuses of the inner layer and detection of capillary abnormalities in the early stage of diseases that may not be visible in combined inner retinal angiography. By using these techniques on diabetic patients without clinical signs of retinopathy, we clearly showed that the microvascular changes in the deep retinal capillary layer could be detected before early-stage retinopathy was evident. A prospective study with a larger cohort is needed to verify this.

There are several limitations in the current study. First, although the OCT-A provided improved visualization of the superficial and deep capillary networks compared to FA, the current technology of OCT-A is limited by a smaller field of view than is provided by FA. This may limit our understanding of the vascular changes in the peripheral retina in the early stage of DR. Second, the BG and HbA1C of the control participants were not examined in the study. Therefore, it cannot be excluded that some of the controls had undetected type 2 DM. However, it may not impact the results because the nondiagnosed DM in the controls would most likely have led to an underestimate of the difference in microvascular fractal dimensions between the diabetic patients and the controls instead of an overestimate. In addition, due to the cross-sectional, noninterventional design of the study, it was not possible to make a conclusion that whether the microvascular changes in diabetic patients found in the current study increase the risk of DR development. Further studies to longitudinally follow diabetic patients and to determine the relationships between the microvascular changes found in the current study and other impact factors (i.e., HbA1C, diabetic duration) are necessary to further determine the utility of the fractal analysis from OCT-A.

In conclusion, our results indicated that fractal dimensions decrease in patients with type 2 DM with no DR and in patients with mild DR. The current study also revealed that fractal dimensions in the deep retinal capillary layer had higher discriminating power to assess vascular alteration in early-stage retinopathy, which may constitute an early indicator of retinopathy. Fractal analysis based on OCT-A images offers a new path of study and will likely be useful in the future as an objective biomarker for predicting DR in patients with type 2 DM and early-stage DR that are at risk for progression into fully developed retinopathy.

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