Characteristics of Retinal Structural and Microvascular Alterations in Early Type 2 Diabetic Patients

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Purpose. To investigate early retinal structural and microvascular changes in patients with type 2 diabetes mellitus (DM) and to analyze relationships among the retinal structure, microvasculature, and choroid.

Methods. Seventy-seven patients with type 2 DM (40 with no diabetic retinopathy [DR], 37 with nonproliferative diabetic retinopathy [NPDR]), and 34 control subjects were enrolled. Spectral-domain optical coherence tomography, operating in radial 18-line mode, obtained macular images of the eight intraretinal layers and the choroid. The same system was equipped with Angiovue to obtain angiography images of the whole, superficial, and deep retinal capillary layers (WRCL, SRCL, and DRCL) in a 3-mm-diameter area around the macula. Algorithms quantified the thicknesses of the intraretinal layers and choroid as well as fractal dimensions (Dbox values) of the retinal capillary layers. Pearson’s correlation was used to analyze the relationships.

Results. The choroidal thickness was significantly decreased in all the regions of the DM patients with no DR (P < 0.05). Compared to controls, the Dbox values of the SRCL and DRCL were significantly decreased in diabetic patients with no DR, however, only the nerve fiber layer in this group was slightly thinner than in the controls (P < 0.05). In the two diabetic groups, there was a weak correlation between the ganglion cell complex thickness and the SRCL (P < 0.05).

Conclusions. In DM, changes of retinal microvasculature might occur earlier than changes in retinal structure. Thinning of the choroid may be the earliest sign in the diabetic patients with no clinical DR.

Keywords: retina, thickness, microvasculature, fractals, optical coherence tomography angiography, diabetic mellitus
quickly obtains high-resolution images of the in vivo retinal microvasculature, allowing in-depth visualization of the retinal microvascular network in the different retinal layers.\textsuperscript{17–19} In our previous study,\textsuperscript{20} we have demonstrated that OCT-A and fractal analysis could detect retinal microvascular decreases in diabetic patients even before clinical retinopathy occurs. The goals of the current study were to investigate the early retinal structural and microvascular changes as well as changes in the choroid, which supplies nutrition to the outer retina, in a cohort of type 2 diabetic patients with no clinical DR.

**Materials and Methods**

**Subjects**

Most of the patients with type 2 DM were diagnosed by an endocrine specialist (CW) from the endocrinology department of the Second Affiliated Hospital & Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou, China. Some patients with moderate to severe nonproliferative diabetic retinopathy (NPDR) were from the fundus department at the Eye Hospital of Wenzhou Medical University. Age- and sex-matched control subjects were recruited from workers at the Eye Hospital of Wenzhou Medical University and family members of patients at the same hospital. This study was approved by the ethics committee of the Eye Hospital of Wenzhou Medical University. The ethics committee approved the screening, inspection, and data collection, and all patients provided written informed consent. All experiments followed the provisions of the Declaration of Helsinki.

All diabetic patients underwent a series of ophthalmologic examinations, including refraction and best corrected visual acuity (BCVA) test, slit-lamp biomicroscopy, axial length (AL) measurement, intraocular pressure (IOP) measurement, and ophthalmoscopy. Inclusion criteria of the diabetic patients consisted of the diagnosis of type 2 DM with or without NPDR, as determined by the retinal specialists, according to the International Clinical Diabetic Retinopathy Disease Severity Scale.\textsuperscript{21} Exclusion criteria were as follows: refractive errors over \(\pm 2.00\) diopters (D) or under \(-6.00\) D of spherical equivalent (SE) or \(-1.50\) D of astigmatism, significant media opacities, IOP > 21 mm Hg or previous diagnosis of glaucoma, uveitis, or retinal disease, as well as clinically visible macular edema and proliferative DR. Demographic information of all diabetic patients was collected, including age, sex, body mass index (BMI), mean artery pressure (MAP), duration of DM, as well as laboratory serum biochemical indicators, like blood glucose (BG), HbA1c level, triglyceride (TRIG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The control individuals underwent the same tests as those used to evaluate the diabetic patients, except for laboratory serum biochemical indicators.

**OCT Procedure and Data Collection**

The eyes of all enrolled subjects were imaged by an SD-OCT system (Optovue RTVue XR Avanti; Optovue, Inc., Fremont, CA, USA) equipped with Angiovue for OCT-A. The bimodal system operated in the radial scan mode (8-mm diameter; 18 lines) to generate three-dimensional (3D) thickness maps (Fig. 1A). It also operated in the OCT-A mode (3 × 3 mm area) to obtain microvascular images (Figs. 1B–D) of the whole, superficial, and deep retinal capillary layers (WRCL, SRCL, and DRCL) around the macula.

As described previously,\textsuperscript{6,22} each cross-sectional image that included the retina and choroid was analyzed by one masked reader (YW) to segment the 10 boundaries of the intraretinal layers and the choroidal layer. The segmentation was achieved by a dedicated software program based on the gradient information and shortest path search that was developed in Matlab (The MathWorks, Inc., Natick, MA, USA) for automated image analysis. The thickness of each retinal layer was calculated by subtracting the boundary positions of each of the adjacent layers obtained by automated segmentation along the depth. The intraretinal layers included the (1) nerve fiber layer (NFL), (2) ganglion cell layer and the inner plexiform layer (GCL+IPL), (3) inner nuclear layer (INL), (4) outer plexiform layer (OPL), (5) Henle fiber layer + outer nuclear layer (HNL+ONL), (6) the myoid and ellipsoid zone (MEZ), (7) photoreceptor outer segments (OS), (8) interdigitation zone + retinal pigment epithelium (IZ+RPE), and (9) choroid (Fig. 1A).

To match the areas of retinal structure and microvasculature, a 3D thickness map of the intraretinal layers in each eye was generated from the segmented layers in a 3-mm-diameter circle around the macula (Fig. 1E). For analysis, the macular thickness map was divided into five regions and displayed as a 3-mm-diameter circle centered over the fovea and surrounded by a concentric ring 0.5 to 1.5 mm from the fovea. The areas of the central circle and concentric ring were then divided into five regions including the central region (C), the superior, temporal, inferior, and nasal regions (S, T, I, and N, respectively). The mean thicknesses for the total retina and the eight intraretinal layers as well as the choroid in each region were calculated. Repeatability tests were performed for the choroidal thickness measurements in 10 DM eyes and 10 healthy eyes. We found that the standard deviation (SD) of the differences between the two manual segmentations by one masked reader (YW) ranged from 18.5 to 36.7 \(\mu\)m, and the interclass correlation coefficient (ICC) ranged from 0.857 to 0.971 in the DM eyes. However, in the healthy eyes, the SD ranged from 32.0 to 64.6 \(\mu\)m, and the ICC ranged from 0.474 to 0.899.

The OCT-A image size was 304 \(\times\) 304 pixels, and a set of scans with high quality of signal strength index \(> 40\) was selected for further analysis. The WRCL was used from the inner limiting membrane to the RPE, excluding data from beneath the RPE (Fig. 1B). The SRCL extended from 3 \(\mu\)m below the internal limiting membrane to 15 \(\mu\)m below the inner plexiform layer (IPL), which supplies nutrition for the layers of the NFL and GCL+IPL (Fig. 1C). The DRCL, which supplies nutrition for INL and OPL, extended from 15 to 70 \(\mu\)m below the IPL (Fig. 1D).

To quantify the complexity and density of the retinal microvascular network in the OCT-A images, we used a custom automated processing algorithm that included a correction using Bennett’s formula for image magnification based on the AL.\textsuperscript{20,23} In brief, the relationship between OCT image measurements and actual scan diameter was expressed by the formula \(t = p \times q \times x\), where \(t\) represented the actual scan length, \(p\) represented the magnification factor determined by the OCT imaging system camera, \(q\) represented the magnification factor in relation to the eye, and \(x\) represented the original measurement value obtained from the OCT image. The correction factor \(q\) was expressed by the equation \(q = 0.01306 \times (AL − 1.82)\). After image processing, we performed fractal analysis on the skeletonized images of the WRCL, SRCL, and DRCL. The quantitatively measured parameter of fractal dimension, \(D_{box}\), was obtained by using the fractal analysis toolbox from Benoît (TruSoft Benoît Fractal Analysis Toolbox; TruSoft International, Inc., St. Petersburg, FL, USA). The fractal dimension was calculated for the 2.5-mm-diameter total annular zone (TAZ) after excluding the FAZ (diameter = 0.6 mm, Fig. 1F). Fractal dimensions were then automatically calculated in the parafoveal quadrant regions (i.e., S, T, I, and N).
regions of the 2.5-mm-diameter circular zone after excluding the FAZ).

Statistical Methods

All data were expressed as the means ± SDs and were analyzed with SPSS software (version 22.0; SPSS, Inc., Chicago, IL, USA). 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 NPDR. Post hoc tests were used for group pairs. Differences between sexes within each of the three groups were determined by the χ² test. Pearson’s correlation was used to analyze the relationships among the intraretinal thicknesses, choroidal thickness, and the fractal dimensions of the retinal microvasculature. A value of \( P < 0.05 \) was considered statistically significant.

RESULTS

Patient Characteristics

A total of 77 consecutive patients with type 2 DM (40 with no DR; 37 with NPDR) and 34 control subjects were included. One eye of each subject was selected according to a randomization table. Of the 37 eyes with NPDR, 22 had mild NPDR, 12 had moderate, and 3 had severe NPDR. Except for the BCVA (\( P = 0.024 \); Table 1), there were no significant differences among the three groups in age, sex, BMI, MAP, SE, AL, or IOP (\( P = 0.057 \) ~ 0.949). The duration of DM was greater for the NPDR patients than for patients with no DR (\( P = 0.042 \); Table 1), while the TC level was lower in the NPDR patients than in those with no DR (\( P = 0.039 \)). Differences in BG, HbA1c, TRIG, HDL-C, or LDL-C for the two groups were not significant (\( P = 0.181 \) ~ 0.968).

Intergroup Differences in the Raw OCT and OCT-A Images

In the segmented OCT images (Figs. 2A–C), the choroid thicknesses of the two diabetic groups were remarkably thinner than that of the controls; however, the subtle changes in retina thickness among the three groups could not be detected easily by the naked eye. OCT-A images in eyes with no DR (Figs. 2E, 2H, 2K) showed that the FAs in the WRCL, SRCL, and DRCL were all larger than those of the controls (Figs. 2D, 2G, 2J). Further, the microvessels seemed to be sparser, which was much more evident in the NPDR eyes (Figs. 2F, 2I, 2L).
Macular Intraretinal Layer and Choroid Thicknesses

Within the inner retinal layers, the NFL thicknesses of patients with no DR and with NPDR were both significantly lower than those of controls in the T and N regions (post hoc tests, \( P < 0.05 \); Table 2). However, there were no significant differences of NFL thicknesses between the two diabetic groups (\( P = 0.460 \sim 0.978 \); Table 2). For the outer retinal layers, in the diabetic patients with NPDR, the MEZ thicknesses in the T and N regions were significantly thinner than in both the diabetic patients with no DR and the controls (post hoc tests, \( P < 0.05 \); Table 2). However, there were no significant differences in MEZ thicknesses between the diabetic patients with no DR and the controls (\( P = 0.387 \sim 0.790 \); Table 2). The choroidal thicknesses of patients with no groups (\( P = 0.460 \sim 0.978 \); Table 2). For the outer retinal layers, in the diabetic patients with NPDR, the MEZ thicknesses in the T and N regions were significantly thinner than in both the diabetic patients with no DR and the controls (post hoc tests, \( P < 0.05 \); Table 2). However, there were no significant differences in MEZ thicknesses between the diabetic patients with no DR and the controls (\( P = 0.387 \sim 0.790 \); Table 2). The choroidal thicknesses of patients with no
DR were significantly decreased in all the five regions of this group compared to the controls (post hoc tests, \( P < 0.05 \); Table 2). However, the choroidal thicknesses of the diabetic patients with NPDR were not decreased compared to the patients with no DR (\( P = 0.385 \sim 0.758 \); Table 2), even though they were still thinner than those in controls in all the regions (\( P < 0.05 \); Table 2).

**Fractal Analysis of the Macular Microvasculature**

For the WRCI, the \( D_{\text{box}} \) value for the T region in patients with NPDR was significantly lower than for the controls (post hoc test, \( P = 0.01 \)). For the TAZ, S, I, and N regions, there were no significant differences in the \( D_{\text{box}} \) values among the three groups (ANOVA, \( P = 0.190 \sim 0.755 \); Table 3). For the SRCL and DRCL, there were significant differences in the \( D_{\text{box}} \) values in almost all regions among the three groups (ANOVA, \( P < 0.05 \); Table 3). The exception was for the T region of the SRCL (\( P = 0.139 \)). In addition, the \( D_{\text{box}} \) value for the TAZ in the SRCL and the \( D_{\text{box}} \) values for the TAZ and I region in the DRCL were significantly lower in diabetic patients with no DR than those in controls (post hoc tests, \( P < 0.05 \); Table 3). Moreover, the \( D_{\text{box}} \) values for the S (\( P = 0.054 \)), T (\( P = 0.198 \)), I (\( P = 0.313 \)), and N (\( P = 0.251 \)) regions in the SRCL and the \( D_{\text{box}} \) values for the S (\( P = 0.129 \)), T (\( P = 0.153 \)), and N (\( P = 0.063 \)) regions in the DRCL were consistently lower in diabetic patients with no DR than in controls, though none of the differences were statistically significant.
Relationships Among the Retinal and Choroidal Thicknesses and the Microvasculature

We assessed the relationships among the intraretinal thicknesses and the corresponding vasculature. Only the ganglion cell complex (GCC, including the NFL/GCL/IPL) of both diabetic groups was positively correlated with the Dbox values of the SRCL in the S (r = 0.289, P = 0.011; Fig. 3A) and I regions (r = 0.262, P = 0.021; Fig. 3B). There were no significant correlations between the choroidal thickness and any of the outer intraretinal layers (P = 0.141 ~ 0.872).

**DISCUSSION**

There are two major competing theories regarding the pathogenesis of DR. One supports the microvascular-origin theory, while the other supports the neurodegenerative-origin theory. The resolution of the conflicting theories depends upon determining whether or not the retinal neurodegeneration found in the early stage of DR is a primary and independent change or, instead, a consequence of retinal vascular impairment. To provide evidence relevant to these theories, we performed the current study to investigate characteristics of the in vivo intraretinal layer thicknesses, multiple microvascular layers, and choroid thickness in a cohort of type 2 DM patients without clinical DR. We found that the choroidal thickness was significantly decreased in patients with early-stage DM but with no DR. However, there were no significant changes in the thicknesses of the total retina or intraretinal layers in these patients, except for the NFL in the T and N regions. Moreover, we found that the fractal dimensions of both the SRCL (TAZ) and the DRCL (TAZ and I region) were diminished in diabetic patients with no DR.

Thus, the changes in the vasculature, including the retinal microvasculature and choroid, were more evident than the retinal structure in diabetic patients without clinical DR. Over the past decade of OCT technology development, the morphology of the intraretinal layers has been discerned with high resolution. These advances have shown that retinal neurodegeneration exists even in the preclinical stage of DR, which is an important and early component of the retinopathy. Based on such results, it appeared that the retinal neurodegeneration of DM may occur before the microvascular changes. However, those studies used color fundus photography or biomicroscopy to identify the diabetic patients with no DR. These methods have relatively low resolution and can only visualize large-caliber retinal vessels. Because DR clearly has a microvascular component, it is essential to detect subtle changes to determine if the microvascular impairment already exists in the early stage of DM.

In our previous study, we have used OCT-A to investigate the fractal characteristics of the retinal microvessels and found that the complexity and density of the vasculature are already decreased in patients with type 2 DM before the clinical signs of DR occur. This finding was validated again in this study. It strongly indicated that fractal analysis from OCT-A images can identify preclinical lesions of the retinal capillaries in DM before the clinical appearance of retinopathy. Similar observations have also been reported by Di et al. and Takase et al. who found the enlargement of FAZ by OCT-A.

While alterations in the retinal vasculature that result in compromise of the blood-retinal barrier play a critical role in the pathophysiology of DR, changes in the underlying choroidal vasculature may also have a contributing role. Histopathologic and animal studies have reported choroidal vascular degeneration, choroidal aneurysms, choriocapillaris dropout, choroidal neovascularization, blood flow change, and increased tortuosity and narrowing of the vessels in the development of DR. Moreover, the previous studies indicate that diabetic patients have a thinner choroid and subsequent retinal tissue hypoxia, regardless of the disease stage, probably due to choriocapillaris dropout caused by hyperglycemia and hypoinsulinemia. In recent years, the development of new OCT technologies and imaging software has made it possible to provide more detailed images of the choroidal anatomy and topography. In this study, we used SD-OCT and found that even though the changes of the intraretinal layers were not obvious, the choroidal thicknesses in all five of the analyzed regions were significantly decreased both in the diabetic patients with no clinical DR and with NPDR, which was consistent with the findings of Tavares et al. and Pierro et al. However, we did not find that the choroidal thickness in...
the patients with NPDR continued to decrease compared to patients with no DR. There could be two reasons for this. First, significant individual variability in the two diabetic groups existed and could have obscured any continuous but subtle decreases in thickness. Second, the choroidal thickness was reported to be decreased in eyes with no DR and in mild to moderate NPDR eyes, but increased in eyes with severe NPDR and PDR. For the severely affected eyes, increased vascular endothelial growth factor or cytokines mediating choroidal vasodilation and blood flow elevation might be responsible for the apparent increase in choroidal thickness. In our study, we enrolled only three severe NPDR eyes, which was too few to support a statistically significant increase in the thickness of the choroid.

In the current study, we found that only the NFL thickness was decreased in diabetic patients with no clinical DR, but not the total retina and other intraretinal layers. Among recent studies, most have not found thickness changes of the total retina in similar patients. Exceptions are reported by Bronson-Castain et al. who have found that the total retinal thickness is decreased in patients with no DR. Sohn et al. report a progressive loss of NFL thickness (0.2 μm/y) and GCL-IPL thickness (0.29 μm/y) in the early stage of DR. Some authors also have found that the NFL around the optic disc is significantly thinner in diabetic patients with no DR. However, Van Dijk et al. and Park et al. have not found significant thickness changes in the macular NFL or the GCL-IPL in patients with no DR, but the GCL-IPL is thinner in patients with minimal DR. In the outer intraretinal layers, we found that only the MEZ, including the inner segments (IS) of the photoreceptors, was significantly thinner in the patients with NPDR. It is possible that the vasculopathy of choriocapillaris, which nourishes photoreceptors, may contribute to the photoreceptor degeneration. An animal study indicates that the MEZ is the only layer that consumes O2, and the intraretinal oxygen tension is lowest in this layer compared to the OS and the HFL+ONL. Therefore, the IS of photoreceptors are the most sensitive to hypoxia in the development of DR and likely responsible for the early thinning of the MEZ.

We further analyzed the relationships between the intraretinal layer thicknesses and the corresponding fractal dimensions of the vascular layers that supply them in parafoveal areas of similar size. We found only a weak correlation (r = 0.262 ~ 0.289) between the GCC and the SRCL in the two diabetic groups. This was consistent with the findings of Frydkjaer-Olsen et al. who also have found a weak correlation (r = 0.20) between the GCL thickness and the retinal fractal dimension of color fundus photographs in patients with no or minimal DR. In addition, they also report an independent association of retinal vessel caliber with the neuroretinal changes in patients with no or early DR. Even though we found a small correlation of the inner retinal layer thickness and the fractal dimension of the SRCL, this did not clearly eliminate the doubt of whether or not the retinal neurodegeneration developed independently or was affected by the damaged retinal vasculature. Resolving this issue will require a future longitudinal follow-up study with a larger sample size.

In our present study, we used SD-OCT to image the choroid, as reported in some previous studies. The high-resolution images enabled good visualization of the choroid in diabetic patients, and the excellent ICCs enabled the reliable measurement of thickness changes at each choroidal location. In healthy subjects, the choroid-scleral interface is not as clear as that in the diabetic patients owing to the sensitivity drop-off. Therefore, we used manual segmentation to check the choroidal thickness. The ICC was slightly lower for the choroidal measurement in healthy subjects than in the diabetic patients, but the repeatability was still within the range reported in previous studies. However, this may not have had an impact on the current results because the manual detection of the choroid-scleral interface in the healthy patients would most likely have led to an underestimate of the difference in choroidal thickness between diabetic patients and controls instead of an overestimate. A prospective study using swept-source OCT or SD-OCT with enhanced depth imaging is needed to verify this.

There were several limitations in the current study. First, the analyzed retinal area was limited by the parafoveal region (2.5 ~ 3 mm around the fovea). Perhaps some significant information in a larger field was lost. Second, the heterogeneity existed in the NPDR group, as we excluded many moderate or severe patients with clinically visible macular edema. However, the current study mainly focused on the NDR patients (diabetic patients with no DR) who were considered to be in the earliest stage of retinopathy. We will report in more depth on the NPDR group in future longitudinal studies regarding the
development of the retinal structural and the microvascular damage from mild to severe conditions. Third, a commonly acknowledged technical limitation of OCT-A remains the inclusion of projection artifacts, particularly in the deep layer of OCT-A images. Additionally, because the manufacturer’s software does not always detect the true boundary between the IPL and the INL, incorrect segmentations of both the superficial and deep layers in the OCT-A or en face OCT images frequently occur in diseased eyes. Therefore, the fractal dimension in the deep layer could be affected by segmentation errors in addition to the projection artifacts.

In conclusion, significant thinning of the choroidal thickness both in the fovea and parafovea may be the earliest sign of the early diabetic stage with no clinical DR. Fractal analysis based on OCT-A images is a potentially powerful method to detect subtle changes of the retinal microvasculature in DM. These changes might occur earlier and be more profound than the early changes in retinal structure. This hypothesis will be further tested by a longitudinal study in the future.

Acknowledgments

The authors thank Britt Bromberg, PhD, of Xenofile Editing (www.xenofileediting.com) for providing editing services for this manuscript.

Support by research grants from the National Science Foundation of China (Grant No. 11675122, No.81570880), the Public Service Program of Wenzhou Science and Technology Bureau of China (Y20160151, Y20160450), the National Key Project of Research and Development Program of China (2016YFE0107000, 2016YFC0102500), 111 Project (D16011), and Zhejiang Provincial Key Research and Development Program Grant (2015C03029).

Disclosure: Q. Chen, None; F. Tan, None; Y. Wu, None; X. Zhuang, None; C. Wu, None; Y. Zhou, None; Y. Li, None; D. Cheng, None; J. Wang, None; F. Lu, None; M. Shen, None

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