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Quantitative analysis of macular capillaries in diabetic patients using optical coherence tomography angiography

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光学相干断层扫描血管成像量化分析糖尿病患者黄斑区毛细血管参数

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摘要

目的:应用光学相干断层扫描血管成像技术(OCTA)量化2型糖尿病患者黄斑区毛细血管的早期变化。

方法:回顾性病例研究。分别纳入49名健康受试者、52例无视网膜病变的2型糖尿病患者(noDR)和43例轻度非增殖性糖尿病视网膜病变(mNPDR)患者,并得到在黄斑区3 mm×3 mm浅层毛细血管丛和深层毛细血管丛的OCTA图像。去除大血管后分别计算毛细血管灌注密度、血管长度密度(VLD)和平均血管直径(AVD)并进行比较。应用受试者工作特征曲线评估该参数监测2型糖尿病患者视网膜微血管早期改变的能力。

结果:比较三组间VLD和AVD,差异均有统计学意义($P<0.001$)。与健康受试者相比,noDR组的AVD均显著增加($P<0.05$)。mNPDR组患者深层及浅层的VLD较noDR组显著下降(均$P<0.01$)。深层AVD鉴别noDR组与健康受试者的曲线下面积(AUC)为0.796,鉴别mNPDR组和健康受试者的AUC最高为0.920,其次为深层VLD(AUC=0.899),显著高于其他参数。

结论:在糖尿病视网膜病变的临床前阶段,2型糖尿病患者的深层及浅层AVD均显著高于健康人,VLD均显著高于mNPDR患者。与健康人相比,深度AVD较其他参数更能检出noDR患者早期视网膜毛细血管的变化。

关键词:糖尿病视网膜病变;光学相干断层扫描血管成像(OCTA);灌注密度;血管长度密度;血管直径

Abstract

• AIM: To quantify early changes of macular capillary parameters in type 2 diabetic patients using optical coherence tomography angiography (OCTA).

• METHODS: Retrospective case study. A total of 49 healthy subjects, 52 diabetic patients without retinopathy (noDR) patients, and 43 mild nonproliferative diabetic retinopathy (mNPDR) patients were recruited. Capillary perfusion density, vessel length density (VLD), and average vessel diameter (AVD) were calculated from macular OCTA images (3 mm×3 mm) of the superficial capillary plexus after segmenting large vessels and the deep capillary plexus. Parameters were compared among control subjects, noDR, and mNPDR patients. The area under the receiver operating characteristic curve estimated the abilities of these parameters to detect early changes of retinal microvascular networks.

• RESULTS: Significant differences were found in the VLD and AVD among the three groups ($P<0.001$). Compared with the control group, the noDR group had significantly higher AVD ($P<0.05$). VLD of both layers in patients of mNPDR group was significant decreased compared with that of noDR group (all $P<0.01$). Deep AVD had a higher area under the curve (AUC) of 0.796 than other parameters to discriminate the noDR group from the healthy group. Deep AVD had the highest AUC of 0.920, followed by that of the deep VLD (AUC = 0.899) to

discriminate the mNPDR group from the healthy group.
• CONCLUSIONS: NoDR patients had wider AVD than healthy individuals and longer VLD than mNPDR patients in both layers. When compared with healthy individuals, deep AVD had a stronger ability than other parameters to detect early retinal capillary impairments in noDR patients.
• KEYWORDS: diabetic retinopathy; optical coherence tomography angiography (OCTA); perfusion density; vessel length and density; vessel diameter
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INTRODUCTION

Diabetes mellitus (DM) affected 463 million people globally in 2019 and is likely to continue increasing considerably. Among all diabetes, type 2 DM (T2DM) was accounting for 90%[1]. It progressively impairs the retinal microvascular structure in 35% of patients with DM, resulting in diabetic retinopathy (DR), which remains a leading cause of visual impairment and blindness in the world field[1-2]. Determination of retinal microvasculature changes at an early stage will identify the pathophysiology of DR and provide timely recognition and management for patients at a greater risk of DR progression. Although defective blood flow is an early indicator of retinal dysfunction during DM, it cannot be clinically indicated. Patients with defective blood flow will continue to be classified as normal until the appearance of visible lesions[3].

As a noninvasive fundus angiography method, optical coherence tomography angiography (OCTA) can quantitatively and quickly measure the retinal microvasculature in various layers. It therefore becomes widely used to assess compromised microvasculature in DM patients without DR. One of the most studied parameters to evaluate pre-clinical DR is vessel perfusion density (PD). However, when compared with healthy individuals, divergent conclusions have been reported[4-9], as well as discrepancies between superficial and deep retinal layers[10-13].

The macular retinal capillary containing pericytes and endothelial cells, is the critical interface for the exchange of nutrients, oxygen, and metabolites between the neuropil and the circulation. Besides pericytes and endothelial cells, arterioles also contain smooth muscle cells. A previous study had reported that retinal capillaries and large vessels responded differently to DM[14]. Based on these results, when studying macular vessel plexus, quantification may be grossly inaccurate if capillaries, venules, and arterioles are studied together. The retinal capillary parameters were the main indicators quantified and studied in this paper, so we needed to remove the venules and arterioles before quantification. Because vessel density is determined by vessel length and vessel diameter, investigating how these two parameters change during the pre-clinical DR stage will hopefully resolve the divergence of PD observations of previous investigations. Furthermore, measuring these two parameters can be used to monitor the progression of the condition in DR patients, take appropriate treatment measures, and uncover the pathogenesis of DR. Although these parameters have been described in OCTA studies of DR[4,7-9,15], there were few studies focusing on capillary parameters in pre-clinical DR patients.

In the present study, we used OCTA to separately quantify macular microvascular morphology in the superficial capillary plexus (SCP) and deep capillary plexus (DCP). A series of image processing algorithms were used to enhance and classify the OCTA images, and remove large vessels to retain capillaries. The PD, vessel length density (VLD), and average vessel diameter (AVD) were quantitated from superficial and deep OCTA images. We then determined how these parameters varied in healthy and T2DM subjects, and demonstrated the abilities of these parameters to accurately detect abnormalities in the retinal capillary network of T2DM patients.

METHODS

Subjects In this cross-sectional and retrospective study, we recruited three cohorts consisting of healthy controls, patients with T2DM without clinical signs of DR (noDR), and patients with mild nonproliferative DR (mNPDR). The mNPDR patients were identified using the second stage of the International Clinical Diabetic Retinopathy Disease Severity Scale[16]. All subjects were recruited from the First Hospital of Qinhuangdao who presented between January to December 2018. The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Institutional Review Board of First Hospital of Qinhuangdao (No. 2019A044). Informed consent was obtained from all participants. Inclusion criteria were as follows: 1) best corrected visual acuity of 20/20 or better and age >18 years, and participants were healthy or with T2DM, as confirmed by a diabetologist; 2) the DM patients were diagnosed with no more than mNPDR and without diabetic macular edema; 3) no history of other ocular diseases or significant media opacity, no previous intraocular treatment (laser, intravitreal injections, cataract surgery, or vitreoretinal surgery), refractive error < 4 diopters; 4) without ischemic heart disease, hypertension or neurodegenerative disease; 5) eyes with good-quality images on OCTA (quality index higher than 30) that did not confound the analyses. We selected the eye with better OCTA image quality if both eyes of one participant correlated with the inclusion criteria.

Clinical Parameters For both control and DM patients, a series of examinations were performed, including best

corrected visual acuity, intraocular pressure (IOP), refractive error, and slit lamp fundus examination. The retina was evaluated and graded by an experienced ophthalmologist according to the slit lamp fundus examination results during mydriasis with a 90-D lens and 35° 7-standard field color retinal photographs[17]. The blood pressure, body mass index (BMI), glycated hemoglobin (HbA1c) levels, and duration of diabetes were also determined.

The Heidelberg Spectralis OCT2 OCTA instrument was used in this study. An OCTA scan pattern was 3 mm × 3 mm (consisting of 512 B-scans) centered on the macula and the automatic image intensity score was set at +4. The OCTA volume was automatically segmented into the SCP and DCP for macular microvascular measurements using the OCTA software (Heyex Software Version 1.9.201.0; Heidelberg Engineering, Heidelberg, Germany). The SCP was defined as a slab extending from the internal limiting membrane to 17 μm above the inner plexiform layer. The DCP was a slab extending from 17 μm above the inner plexiform layer to 10 μm below the outer plexiform layer. All OCTA angiograms were carefully evaluated to detect potential segmentation errors by two experienced specialists. If there was any discrepancy between the observers, it was discussed with another three specialists to reach a consensus decision. In case of segmentation errors, further manual correction of the segmentation was manually conducted. Central foveal thickness (CFT), average central macular thickness in a 1 mm radium circle (CMT), and the ring-shaped region between a 3-mm circle and 1-mm circle (CMT_{3mm}) were parameters identified in the built-in software of our OCTA instrument and were subsequently analyzed.

The venules and arterioles contained in SCP were needed to be removed first before quantification of the parameters. In this study, large vessels were defined as arcades with their first and second branches according to the Horton-Strahler approach[18]. Capillaries were defined as vessels with an absence of smooth muscle cells and with a diameter less than 10 μm[19]. Because the DCP didn't need to perform this step, we show the quantitation of macular capillary parameters as an example of a superficial OCTA image below (Figure 1). Figure 1A shows the original OCTA image of macular SCP. The size of the image was 900×900 pixels, which covered the macular area of 3 mm×3 mm. We first reduced image noise by using a median filter (5×5) and a Gaussian filter (7×7), and then a Hessian filter was used to enhance capillaries. The enhanced image is shown in Figure 1B. A local adaptive region growth algorithm proposed previously[20] was used to divide the blood vessels. Figure 1C is the binarized blood vessel image obtained according to the segmentation results. A distance transformation operation was performed on the binarized image, as shown in Figure 1D. Each pixel value in Figure 1D represents the nearest distance from the current vascular boundary. The nearer the pixel to the vessel axis, the larger it is. A non-maximum suppression method commonly occurring in the Canny operator was used to obtain the vascular skeleton (Figure 1E). Each pixel point in the skeletal image represents the radius of the current blood vessel. The value in the color bar represents the number of pixel points (the size of each pixel point is 3.33 μm×3.33 μm). We defined blood vessels with a diameter greater than the width of 4 pixels as large vessels and extracted them (Figure 1F), and then the macular superficial capillary image was obtained, as shown in Figure 1G. In Figure 1G, the annular region between the two red circles is the region for capillary parameters quantization. PD is defined as the percentage of vascular pixels that account for all pixels in the image. The skeleton that eliminates the large vessels is the capillary skeleton. VLD was calculated as the percentage of the capillary skeleton pixels that account for all pixels in the image based on the skeletonized OCTA image. The pixel value in the capillary skeleton represents the radius of the capillary. Therefore, AVD can be obtained by multiplying the mean of the capillary skeleton by two.

Data Analysis All the quantitative features for different groups were tested for normality by the Shapiro-Wilk test. For normally distributed variables, we first calculated the means and standard deviations of the main outcome parameters. One-way analysis of variance (ANOVA) was used to test for differences among the three groups, and post hoc tests were used between group pairs. Differences in the duration of diabetes and HbA1c between eyes of no DR and mNPDR groups were analyzed by student's t-test. Nonparametric tests were used if the parameters did not conform to normal distribution. The differences between sex and right/left eye within each of the three groups were determined by the χ^2 test or Fisher's exact test. The above statistical analysis was performed using a commercially available statistical software program (SPSS for Mac, version 25.0; IBM/SPSS, Chicago, IL, USA). The receiver operating characteristic (ROC) curve analysis was calculated to evaluate the ability of the OCTA-based parameters to determine the microvascular network impairments at the pre-clinical DR stage. The significance of the difference between two ROC curves was confirmed by the Delong test for two curves with different parameters. All ROC-related statistical analyses were performed using the MedCalc software (version 20.009-64-bit; https://www.medcalc.org/). A two-tailed P value below 0.05 was considered statistically significant.

RESULTS

A total of 49 eyes from control subjects, 52 eyes from patients with noDR, and 43 eyes from patients with mNPDR were included in the study. There were no significant differences among the control, noDR group, and mNPDR group regarding age, sex, BMI, and systolic blood pressure/ diastolic blood pressure (SBP/DBP). The specific demographic and clinical characteristics of the participants are shown in Table 1.

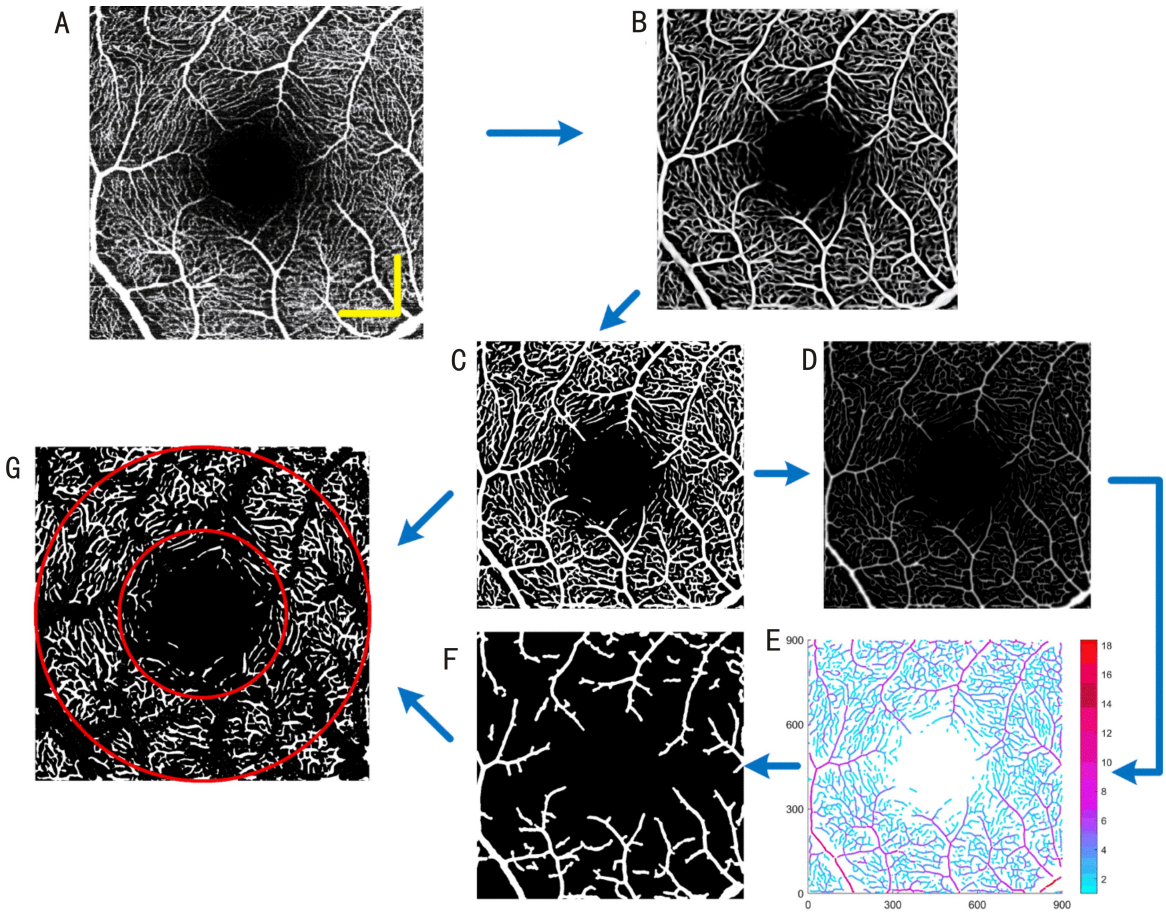

Figure 1 Quantization process of macular capillary parameters. A): Original optical coherence tomography angiography image of macular superficial vascular plexus; B): Capillary enhanced image used by the Hessian filter; C): Binarized image of the superficial vasculature; D): Distance transform image; E): Skeletonized image; F): Large vessels image; G): Macular superficial capillary image. The annular region between the two red circles is the region for capillary parameters quantization.

Table 1 Characteristics of type 2 diabetic patients and controls

Parameters	Healthy group (n=49)	NoDR group (n=52)	mNPDR group (n=43)	P
Age ($\bar{x}\pm s$, years)	53.00±11.31	54.54±10.31	55.49±8.25	0.626[a]
Gender (male/female)	24/25	24/28	23/20	0.775[b]
Eye (right/left)	27/22	29/23	22/21	0.893[b]
Duration of diabetes ($\bar{x}\pm s$, year)	NA	8.08±2.90	8.44±3.53	0.581[c]
HbA1c ($\bar{x}\pm s$, %)	NA	8.84±2.21	9.29±2.26	0.328[c]
SBP ($\bar{x}\pm s$, mmHg)	74.91±7.93	75.35±7.05	77.93±5.58	0.089[a]
DBP ($\bar{x}\pm s$, mmHg)	121.76±11.96	122.01±12.53	126.14±10.43	0.110[a]
BMI ($\bar{x}\pm s$)	23.04±1.54	23.35±1.84	23.77±1.86	0.142[a]

NA: Not applicable; HbA1c: Glycosylated hemoglobin; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; NoDR: Diabetic patients without retinopathy; mNPDR: Mild nonproliferative diabetic retinopathy; [a]One-way analysis of variance; [b]χ^2 test; [c]t-test.

The mean values of OCTA-based parameters and comparison results are listed in Table 2 and partly depicted in Figure 2. All OCTA-based parameters were normally distributed. The increases of CFT, CMT, and CMT_{3mm} all correlated with increases in the severity of DR, however, there was no significant difference among the three groups. Compared with the control group, the noDR group had significantly higher AVD values in both the SCP and DCP layers (P=0.041 and P<0.001, respectively). There were significant differences in the VLD and AVD of both layers and a significantly lower deep PD between the control and mNPDR groups. In addition, compared to the eyes of the noDR group, there were significant differences in PD, VLD, and AVD in the eyes of the mNPDR group in the DCP (P<0.001, P<0.001 and P=0.002, respectively), and significantly lower VLD in the SCP (P=0.005).

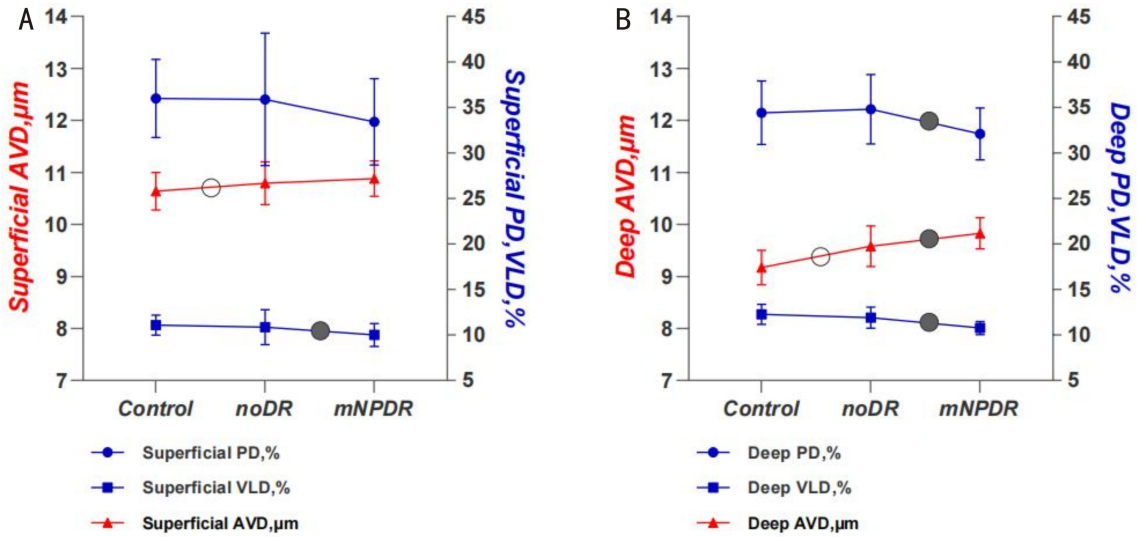

Figure 2 Comparisons of the macular microvascular perfusion density, vessel length density, and average vessel diameter on optical coherence tomography angiography images in the superficial capillary plexus layer (A) and deep capillary plexus layer (B).

"○" represents there was a significant difference between diabetic patients without retinopathy (noDR) group and control group (P<0.05); "●" represents there was a significant difference between noDR and mild nonproliferative diabetic retinopathy (mNPDR) group (P<0.05). AVD: Average vessel diameter; PD: Perfusion density; VLD: Vessel length density.

Table 2 Quantitative analysis results of optical coherence tomography angiography parameters in healthy individuals and patients with noDR and mNPDR $\bar{x}\pm s$

Parameters	Healthy group (n=49)	NoDR group (n=52)	mNPDR group (n=43)	P-value	P^1-value	P^2-value	P^3-value
CFT (μm)	215.31±13.64	220.35±17.24	223.63±21.66	0.089	--	--	--
CMT(μm)	257.47±16.82	259.71±16.97	265.95±24.39	0.103	--	--	--
CMT_{3mm}(μm)	334.38±15.50	333.73±18.72	340.44±21.04	0.170	--	--	--
Superficial PD (%)	35.97±4.29	35.88±7.26	34.31±4.98	0.304	--	--	--
Superficial VLD (%)	11.09±1.10	10.86±1.92	9.97±1.31	0.001	0.431	<0.001	0.005
Superficial AVD (μm)	10.64±0.36	10.79±0.41	10.88±0.37	0.009	0.041	0.003	0.279
Deep PD (%)	34.41±3.48	34.80±3.81	32.25±2.98	0.001	0.599	0.003	<0.001
Deep VLD (%)	12.28±1.10	11.91±1.16	10.75±0.76	<0.001	0.073	<0.001	<0.001
Deep AVD (μm)	9.17±0.33	9.58±0.39	9.81±0.31	<0.001	<0.001	<0.001	0.002

P-value for the comparison among three groups by one-way analysis of variance; P^1-value for the comparison between the diabetic patients without retinopathy (noDR) and healthy groups by post hoc tests; P^2-value for the comparison between mild nonproliferative diabetic retinopathy (mNPDR) and healthy groups by post hoc tests; P^3-value for the comparison between no DR and mNPDR groups by post hoc tests. --: Not performed; CFT: Central foveal thickness; CMT: Average central macular thickness in a 1 mm radium circle; CMT_{3mm}: Average central macular thickness in the ring-shaped region between a 3 mm and 1 mm circle; PD: Perfusion density; VLD: Vessel length density; AVD: Average vessel diameter.

Figure 3 shows the ROC curves of each OCTA metric (only recruited metrics that had significant differences in both analysis of variance and post hoc tests) to discriminate among the control, noDR, and mNPDR groups. The ROC curve analysis of the deep AVD had significantly higher AUCs of 0.796 (95% CI: 0.704 to 0.870, sensitivity = 80.77%, specificity = 69.39%, P < 0.001) compared with that of superficial AVD (AUC = 0.602, 95% CI: 0.499 to 0.698, sensitivity = 32.69%, specificity = 87.76%, P = 0.0732) for differentiating noDR group from control group (P < 0.001; Figure 3A). For discrimination between the mNPDR group from control group, the ROC curve analysis of deep AVD had the highest AUCs of 0.920 (95% CI: 0.844 to 0.966, sensitivity = 86.05%, specificity = 91.84%, P < 0.001), followed by that of deep VLD (AUC = 0.899, 95% CI: 0.819 to 0.952, sensitivity = 90.70%, specificity = 77.55%, P < 0.001). These two parameters had no significant difference from each other (P = 0.677), but were significantly higher compared to AUCs using ROC curve analysis of deep PD (AUC = 0.678, 95% CI: 0.572 to 0.771, sensitivity = 72.09%, specificity = 59.18%, P = 0.009), superficial VLD (AUC = 0.735, 95% CI: 0.633 to 0.822, sensitivity = 51.16%, specificity = 87.76%, P = 0.002), and superficial AVD (AUC = 0.664, 95% CI: 0.558 to 0.759, sensitivity = 48.84%, specificity = 81.63%, P = 0.004), all P < 0.05 (Figure 3B). To discriminate the mNPDR groups from no DR

group, the ROC curve analysis of deep VLD had the highest AUCs of 0.794 (95% *CI*: 0.699 to 0.870, sensitivity = 90.70%, specificity = 63.46%, $P<0.001$), which were significantly larger than that of deep PD (AUC = 0.717, 95% *CI*: 0.615 to 0.805, sensitivity = 72.09%, specificity = 65.38%, $P<0.001$), deep AVD (AUC = 0.683, 95% *CI*: 0.579 to 0.775, sensitivity = 86.05%, specificity = 51.92%, $P<0.001$) and superficial VLD (AUC = 0.667, 95% *CI*: 0.563 to 0.760, sensitivity = 76.74%, specificity = 59.62%, $P=0.003$), all $P<0.05$ (Figure 3C).

DISCUSSION

As early and prevalent targets of DM, subtle changes in retinal microvasculature are thought to be indicators of retinopathy onset and development. This study quantitatively characterized the macular PD, VLD and AVD among healthy, noDR, and mNPDR subjects using OCTA images. In addition to the built-in software, we applied a series of image processing algorithms to enhance and classify microvascular images while removing large vessels to retain capillaries. Furthermore, we quantitated VLD and AVD separately from superficial and deep OCTA images, in addition to vessel PD. Instead of using the vessel diameter index (VDI) in previous studies[4,7,21], we calculated the actual average width of capillaries for analysis.

Previous studies have reported that significant increases in the CMT and average cube thicknesses correlated with an increase in the severity of retinopathy[22-24]. In the present study, we found that during the progression of DR, the values of CFT, CMT, and CMT_{3mm} increased. However, there was no significant difference among the three groups.

Regarding how the macular PD changed in eyes of the noDR patients, it was inconsistent with the results of previous studies when compared with healthy individuals. Most studies reported that macular (whole or part of the regions) PD values decreased during the preclinical DR stage, when compared with healthy individuals[4,8-9,25-27]. These findings suggested that compromised circulation before manifested DR and diffuse capillary nonperfusion were early processes during disease progression. However, Yang *et al*[5] reported that the parafoveal PD in the deep retinal vascular layer was higher in the noDR group. In a study by Thompson *et al*[6], who analysed 37 eyes of 20 DM patients without DR, the PD of the macular cube images using OCTA was significantly increased in patients with microaneurysms (MAs), when compared with patients without MAs. They speculated that the increased vessel density reflected worsening subclinical disease in noDR eyes, which represented the initial stage of underlying retinal tissue hypoxia. In addition, some studies did not detect any quantitative difference in parafoveal vessel density, either in the superficial or deep vessel plexuses of noDR patients[12].

Compared with the PD, the decrease of VLD has been reported to be a more accurate indicator of macular capillary loss in the noDR patients, because the VLD was analyzed in a skeletonized image that removed the influence of vessel size on retinal perfusion measurements[4]. The values of VLD are affected by the OCTA resolution, so we could not compare the VLD directly with other studies. Consistent with previous studies[4,7,21], we confirmed that as diabetes-driven capillary impairments progressed, macular VLD values gradually decreased. However, when comparing the control and noDR groups, there was no significant difference in the VLD. In the present study, the adaptive region growth algorithm dividing the blood vessels automatically adjusted the growth criterion based on the intensity of the vascular signal around the current seed point pixel, which made it especially suitable for the analyses of vascular segmentation from uneven OCTA images.

To investigate changes of capillary calibers in OCTA images, VDI has been used in previous subjects[4,7,21], which was calculated as the nonskeletonized vascular area divided by the skeletonized total vessel length. Previous studies found that increasing VDI was associated with worsening DR[7,21]. Zhu *et al*[10] reported that diabetes made the VDI of retinal capillary

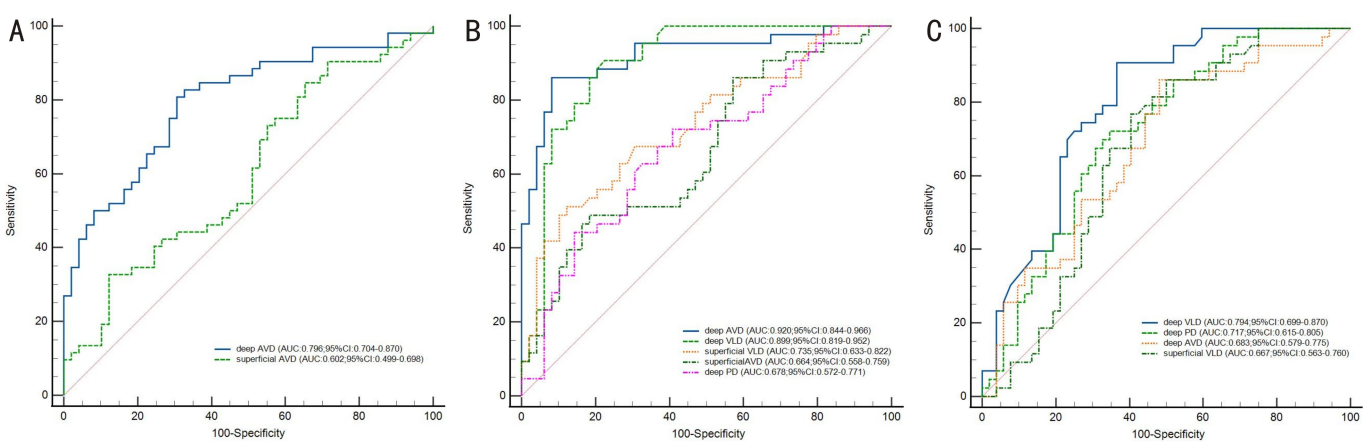

Figure 3 Receiver operating characteristic curves of the optical coherence tomography angiography-based parameters in the task of distinguishing the diabetic eyes without DR group from those of control group (A), distinguishing the mild nonproliferative diabetic retinopathy group from control group (B), and distinguishing the diabetic eyes without DR from the mild nonproliferative diabetic retinopathy group (C). AVD: Average vessel diameter; PD: Perfusion density; VLD: Vessel length density; AUC: Area under curve

networks increased, but the increase was only significant in the DCP. In addition, using ROC curve analyses, it was found that the VDI had weaker discriminating power to assess the diagnostic ability to detect the presence of DR, with AUCs lower than 0.6. In contrast to previous studies, the AVD represented the capillary caliber in this study, which was calculated independently and was not affected by errors in the calculation process of the PD and VLD. We found that macular capillaries were dilated before manifested DR in both SCP and DCP. In addition, when comparing the control and noDR groups, there was no significant difference in the VLD, but a significant increase of the AVD was found in both SCP and DCP. Notably, when comparing the noDR and mild DR groups, there was a significant difference in VLD in both layers. We also found that the deep AVD had a strong discriminating power between the control and mNPDR groups, and a modest discriminating power between the control and noDR groups. This phenomenon suggested that the hyperglycemia-driven macular capillary dilation happened before microvascular nonperfusion in type 2 DM patients.

As the first sign of DR, MAs represent dilations of capillaries, whose diameters vary from 25-100 μm[28]. MAs also account for the increased vessel density and AVD during mNPDR. Our image process methodology eliminated the macular arterioles and venules, as well as MAs in the SCP layer, which led us to underestimate the PD and AVD of the mNPDR group. This could have accounted for the negative results when comparing the AVD values of the SCP between the noDR and mNPDR groups.

The net effect of increasing capillary diameter is an increase in blood flow and thus improved tissue oxygenation. The finding of wider retinal capillaries during diabetes is supported by clinical studies on the effects of diabetes on retinal blood flow and vascular diameters. These studies suggested that hyperglycemia and hypoxia initiated retinal vasodilation of the diabetic retina, leading to hyperperfusion, which interfered with autoregulation, resulting in further vasodilation[14]. We hypothesized that when autoregulation could not supply the metabolic needs, or gradually increased hyperperfusion destroyed capillary endothelial cells, nonperfusion of retinal capillaries associated with DR occurred. Such an increase in capillary diameter may explain why there was no difference in the corresponding occupied surface area measurements between DM and control eyes before manifesting DR. These changes may be reversible, especially when diabetes is effectively controlled. If such phenomena could be detected in a timely manner, it might be possible to slow the progression of the disease through effective interventions.

Zhu *et al*[10] reported that an increase of the VDI in the superficial retina wasn't significant in diabetes patients, but was attributed to distinctive vascular components present between superficial and deep capillary networks. In addition, some previous studies reported that vascular abnormalities were more pronounced in the deep retinal capillary layer[10-13]. The former study found that large vessels extracted from superficial retinal capillary networks responded more slowly than capillaries from hyperglycemia[14]. In the present study, using the AUCs, the DCP parameters did have a better ability to detect early retinal capillary impairments in diabetic patients. Capillaries from different layers differentially responded to hyperglycemia, depending on their specific structures[29]. Removing the larger vessels in OCTA images of SCP only partly eliminated the differences between SCP and DCP.

There were several limitations in the current study. First, a subclinical pathophysiologic response to hyperglycemia, hypertension, inflammation, hypoxia, and endothelial dysfunction is reflected in changes in retinal vascular calibers[30]. However, part of the risk factors, such as lipid profiles and data of smoking habits were not completely collected in the present study. Moreover, although the skeletonization methods used in our study avoided capillary loss to a great extent, VLD values measured after skeletonization will inevitably be lower than the actual size. Concerning the external algorithm applied in this study, a standard calibration should be used to standardize this method and to assess the underestimation of retinal vessel length. Further comparison with parameters calculated from confocal laser scanning microscopy in the same cohort is needed.

In conclusion, after removing large vessels from the SCP image, our results indicated that in both the superficial and deep capillary networks, noDR patients had wider macular capillary caliber than healthy individuals and longer vessel length than mNPDR patients. Moreover, as we didn't detect a significant decrease of VLD in the noDR group when compared with the control group, we speculated that macular capillary dilation occurred before capillary nonperfusion. Among all the parameters investigated in this study, deep AVD exhibited a stronger ability to detect early retinal capillary impairments in noDR patients compared with healthy individuals. Because the evolution of microvascular networks since the diagnosis of DM2 was not recorded in detail, it could introduce bias in detecting changes. Therefore, a further prospective and longitudinal study is needed to validate this possibility.

REFERENCES

[1] International Diabetes Federation. IDF Diabetes Atlas 9th edition. https://diabetesatlas.org/atlas/ninth-edition/

[2] Tan TN, Wong TY. Diabetic retinopathy: looking forward to 2030. Front Endocrinol, 2023,13:1077669.

[3] Boned-Murillo A, Albertos-Arranz H, Diaz-Barreda MD, et al. Optical coherence tomography angiography in diabetic patients: a systematic review. Biomedicines, 2021,10(1):88.

[4] Zhang BL, Chou YY, Zhao XY, et al. Early detection of microvascular impairments with optical coherence tomography angiography in diabetic patients without clinical retinopathy: a meta-analysis. Am J Ophthalmol, 2021,222:226-237.

[5] Yang JY, Wang Q, Yan YN, et al. Microvascular retinal changes in

pre-clinical diabetic retinopathy as detected by optical coherence tomographic angiography. Graefes Arch Clin Exp Ophthalmol, 2020,258(3):513-520.
[6] Thompson IA, Durrani AK, Patel S. Optical coherence tomography angiography characteristics in diabetic patients without clinical diabetic retinopathy. Eye, 2019,33(4):648-652.
[7] Tang FY, Ng DS, Lam A, et al. Determinants of quantitative optical coherence tomography angiography metrics in patients with diabetes. Sci Rep, 2017,7(1):2575.
[8] Forte R, Haulani H, Jürgens I. Quantitative and qualitative analysis of the three capillary plexuses and choriocapillaris in patients with type 1 and type 2 diabetes mellitus without clinical signs of diabetic retinopathy: a prospective pilot study. Retina, 2020,40(2):333-344.
[9] Dimitrova G, Chihara E, Takahashi H, et al. Quantitative retinal optical coherence tomography angiography in patients with diabetes without diabetic retinopathy. Invest Ophthalmol Vis Sci, 2017,58(1):190-196.
[10] Zhu TP, Li EH, Li JY, et al. Comparison of projection-resolved optical coherence tomography angiography-based metrics for the early detection of retinal microvascular impairments in diabetes mellitus. Retina, 2020,40(9):1783-1792.
[11] Scarinci F, Nesper PL, Fawzi AA. Deep retinal capillary nonperfusion is associated with photoreceptor disruption in diabetic macular ischemia. Am J Ophthalmol, 2016,168:129-138.
[12] Lee H, Lee M, Chung H, et al. Quantification of retinal vessel tortuosity in diabetic retinopathy using optical coherence tomography angiography. Retina, 2018,38(5):976-985.
[13] Chen Q, Ma QK, Wu CM, et al. Macular vascular fractal dimension in the deep capillary layer as an early indicator of microvascular loss for retinopathy in type 2 diabetic patients. Invest Ophthalmol Vis Sci, 2017,58(9):3785-3794.
[14] Lei JQ, Yi EH, Suo Y, et al. Distinctive analysis of macular superficial capillaries and large vessels using optical coherence tomographic angiography in healthy and diabetic eyes. Invest Ophthalmol Vis Sci, 2018,59(5):1937.
[15] Vujosevic S, Muraca A, Gatti V, et al. Peripapillary microvascular and neural changes in diabetes mellitus: an OCT-angiography study. Invest Ophthalmol Vis Sci, 2018,59(12):5074-5081.
[16] Wilkinson CP, Ferris FL, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology, 2003,110(9):1677-1682.
[17] Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology, 1991,98:786-806.
[18] Yu PK, Balaratnasingam C, Cringle SJ, et al. Microstructure and network organization of the microvasculature in the human macula. Invest Ophthalmol Vis Sci, 2010,51(12):6735-6743.
[19] Yamamoto K, Tanimura K, Watanabe M, et al. Construction of continuous capillary networks stabilized by pericyte-like perivascular cells. Tissue Eng Part A, 2019,25(5-6):499-510.
[20] Ma ZH, Ding N, Yu Y, et al. Quantification of cerebral vascular perfusion density via optical coherence tomography based on locally adaptive regional growth. Appl Opt, 2018,57(35):10117-10124.
[21] Kim AY, Chu ZD, Shahidzadeh A, et al. Quantifying microvascular density and morphology in diabetic retinopathy using spectral-domain optical coherence tomography angiography. Invest Ophthalmol Vis Sci, 2016,57(9):OCT362-OCT370.
[22] Saxena S, Caprnda M, Ruia S, et al. Spectral domain optical coherence tomography based imaging biomarkers for diabetic retinopathy. Endocrine, 2019,66(3):509-516.
[23] Phadikar P, Saxena S, Ruia S, et al. The potential of spectral domain optical coherence tomography imaging based retinal biomarkers. Int J Retina Vitreous, 2017,3:1.
[24] Ahuja S, Saxena S, Meyer CH, et al. Central subfield thickness and cube average thickness as bioimaging biomarkers for ellipsoid zone disruption in diabetic retinopathy. Int J Retina Vitreous, 2018,4:41.
[25] Zeng YK, Cao D, Yu HH, et al. Early retinal neurovascular impairment in patients with diabetes without clinically detectable retinopathy. Br J Ophthalmol, 2019,103(12):1747-1752.
[26] Park YG, Kim M, Roh YJ. Evaluation of foveal and parafoveal microvascular changes using optical coherence tomography angiography in type 2 diabetes patients without clinical diabetic retinopathy in South Korea. J Diabetes Res, 2020,2020:6210865.
[27] Hsiao CC, Hsu HM, Yang CM, et al. Correlation of retinal vascular perfusion density with dark adaptation in diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol, 2019,257(7):1401-1410.
[28] Querques G, Borrelli E, Battista M, et al. Optical coherence tomography angiography in diabetes: focus on microaneurysms. Eye, 2021,35(1):142-148.
[29] Sun ZH, Yang DW, Tang ZQ, et al. Optical coherence tomography angiography in diabetic retinopathy: an updated review. Eye, 2021,35(1):149-161.
[30] Betzler BK, Sabanayagam C, Tham YC, et al. Retinal vascular profile in predicting incident cardiometabolic diseases among individuals with diabetes. Microcirculation, 2022,29(4-5):e12772.