ORIGINAL RESEARCH

The Association of Axial Length with Macular Microvascular Changes in Chinese Diabetic Retinopathy Patients

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Objective: To use optical coherence tomography angiography (OCTA) to compare macular blood flow density, subfoveal choroidal thickness (SFCT) and outer retina thickness (ORT) in non-proliferative diabetic retinopathy (NPDR) patients with different axial length (AL).

Methods: Total 42 patients with NPDR with different eye axis were divided into three groups: group A: 22 mm \leq AL \leq 24 mm; group B: 24 mm \leq AL \leq 26 mm; group C: AL \geq 26 mm. Superficial capillary plexus (SCP) in the macular area, vascular length density (VLD) and vascular perfusion density (VPD) in the foveal region, the parafoveal region, the perifoveal region and whole macular region were analyzed. The correlations among axial length, macular microvascular density, SFCT and outer retinal thickness (ORT) were analyzed. **Results:** Compared with group A and B, VLD and VPD in group C were significantly lower except the foveal region, and VLD and VPD were negatively correlated with AL. The difference in SFCT among group A, B and C was significant, and SFCT was negatively correlated with AL. Compared with group A, parafoveal ORT in group C was significantly lower than that in group A, and parafoveal ORT was negatively correlated with AL.

Conclusion: In NPDR patients with different AL, macular microvascular density, SFCT, and parafoveal ORT decreased with the increase of AL.

Keywords: diabetic retinopathy, axial length, optical coherence tomography angiography, retinal vascular density, choroidal thickness

Introduction

Diabetic retinopathy (DR) is an eye disease that may lead to blindness.¹ The macula is the most sensitive area of vision, and the changes in the area of the foveal avascular zone (FAZ) can reflect the ischemia of the retina. Studies have shown that the long eye axis is a protective factor for DR, and the mechanism may be related to the changes in retinal blood flow density.^{2–4} However, the retinal and choroidal blood flow density of DR patients with different axial lengths have not been reported. Optical coherence tomography (OCTA) can show macular vascular morphology quickly and non-invasively in real time.^{5,6} In addition, OCTA is not affected by traditional contrast fluorescein leakage and masking and drug allergy. Previous study showed that FAZ enlargement was significantly different between diabetic eyes and healthy eyes.⁷ OCTA can be used to distinguish healthy eyes from diabetic eyes, and dynamically monitor the progression of DR.⁶

Epidemiological studies have shown that myopia with a refractive power of more than -5.0D had a lower risk of DR.⁸ This protective effect may be related to the reduction of retinal blood flow, posterior vitreous detachment, and the regulation of vascular endothelial growth factor (VEGF).^{9,10} Multivariate correlation analysis showed that the severity of DR was negatively correlated with axial length (AL).¹⁰ Bazzazi et al¹¹ reported that in diabetic patients the severity of DR with myopia was lower than those with normal refractive index. Recent studies suggested that low blood perfusion state of high myopia was a protective factor for the development of DR.^{12–14}

This study aimed to employ OCTA to compare macular blood flow density, subfoveal choroidal thickness (SFCT) and outer retina thickness (ORT) in non-proliferative diabetic retinopathy (NPDR) patients with different AL to reveal the microvascular relationship between AL and NPDR.

Methods

Subjects

This cross-sectional study was conducted in accordance with Declaration of Helsinki and was approved by Ethics Committee of the Second Affiliate Hospital of Nanchang University.

Total 53 eyes of 42 NPDR patients with moderate severity of DR who visited the Department of Ophthalmology of the Second Affiliate Hospital of Nanchang University from June 2018 to July 2020 were enrolled and all patients provided written informed consent. The patients were divided into normal group (22–24 mm), long axis group (24–26 mm) and super long axis group (26 mm) according to AL. At the same time, 12 people (15 eyes) were recruited as control subjects with no history of intraocular pathology or major systemic vascular disease.

The exclusion criteria were history of eye surgery and trauma; suffering from eye or serious cardiovascular and blood system diseases except diabetes that may affect eye circulation, such as glaucoma and hypertension; using ocular medication within the past 2 weeks; pathological macular changes including macular hole, macular neovascularization, and macular atrophy.

Ocular Examination

Comprehensive ophthalmic examination was performed, and best-corrected decimal VA was converted to logMAR VA. All OCTA images in the 6×6 mm square centered on the fovea were obtained by Cirrus-5000 SD-OCT-based angiography (Carl Zeiss Meditec Inc., USA). They were divided into three regions: the central area with a diameter of 1 mm, the inner ring area with a diameter of 1–3 mm, the outer ring area with a diameter of 3–6 mm, and the whole 6mm×6mm region with the fovea as the center (nerve fiber layer to the inner boundary of the outer plexiform layer). Vascular length density (VLD) and vascular perfusion density (VPD) of above regions were analyzed. The part between the inner ring and the outer ring was divided into upper side, lower side, nasal side, and temporal side. Four quadrants, were measure to define the blood flow density of each part: (1) Macular fovea: the blood flow density in the circle with a diameter of 1.0 mm; (2) The upper, lower, nasal, and temporal sides of the macular fovea: Blood flow density in the four quadrants between the inner ring and the outer ring; (3) Paracentric fovea: the average blood flow density of the four quadrants; (4) Above and below the macula: passing between the inner ring and the outer ring. The horizontal line of the fovea was the boundary, which was divided into the blood flow density above and below. AL was measured using partial coherence interferometry model 700 (IOLMaster, Carl Zeiss Meditec AG).

Statistical Analysis

The data are expressed as mean±standard deviation (SD). SPSS (SPSS Statistics for Windows Version 22.0, Armonk, NY, IBM Corp) 22.0 software was used for statistical analysis. The data were analyzed using analysis of variance (ANOVA) with Bonferroni correction to evaluate differences among groups, and the correlation was assessed using Pearson's correlation coefficient. P<0.05 was considered significant.

Results

Characteristics of Subjects

In this study, 42 patients (53 eyes) with NPDR of different axis were collected and grouped based on AL. In normal eye axis group A, there were 20 cases and 28 eyes with AL between 22 mm and 24 mm. In long eye axis group B, there were 12 cases and 15 eyes with AL between 24 mm and 26 mm. In extra long eye axis Group C, there were 10 cases and 10 eyes with AL more than 26 mm. In addition, 12 cases (15 eyes) of healthy persons (22 mm \leq AL<24 mm) were selected as the control group.

There were no significant differences in gender, eye type, and age in each group. There was no significant difference in glycosylated hemoglobin among NPDR groups, but the difference in AL was significant (P < 0.001). There was no significant difference in AL between control group and NPDR group A (P=0.732) (Table 1).

Characteristics	Group A	Group B	Group C	Control	P
Gender (Male/Female)	10/10	5/7	5/5	7/5	0.881
Eye (Right/Left)	15/13	8/7	4/6	7/8	0.879
Age (years)	56.1±7.3	55.7±10.7	50.5±8.1	50.7±7.3	0.093
HbAlc (%)	6.4±0.5	6.5±0.6	6.1±0.7	-	0.195
Axial Length (mm)	23.2±0.5	24.6±0.5	27.0±0.8	23.2±0.5	<0.001*

Table I Clinical Characteristics of the Subjects

Note: Data shown as mean± standard deviation; *P<0.05 was considered significant.

Comparison of Blood Vessel Density Between Control Group and NPDR Patients

The control group and group A were compared with the central SCP area, inner ring area, outer ring area and overall VLD of the macula. The VLD of group A in the above four regions were $7.9\pm3.0 \text{ mm}^{-1}$, $17.2\pm1.4 \text{ mm}^{-1}$, $17.9\pm1.1 \text{ mm}^{-1}$, $17.4\pm1.1 \text{ mm}^{-1}$, and the corresponding VLD of the control group were $10.9\pm2.6 \text{ mm}^{-1}$, $19.6\pm1.4 \text{ mm}^{-1}$, $19.9\pm1.4 \text{ mm}^{-1}$, $19.7\pm1.4 \text{ mm}^{-1}$. The VLD of group A was lower than that of the control group in the corresponding area, and the difference was statistically significant (*P*<0.05, Figure 1A).

We compared the central area of the SCP in the macula, the inner ring region, the outer ring region, and the whole macular region in control group and group A. The VPD of group A in the above four areas were 0.179 ± 0.072 , 0.415 ± 0.038 , 0.447 ± 0.026 , 0.432 ± 0.026 , and the corresponding VPD of the control group were 0.246 ± 0.063 , 0.464 ± 0.022 , 0.488 ± 0.026 , 0.475 ± 0.028 . The VPD of group A was lower than that of the control group in the corresponding area, and the difference was statistically significant (*P*<0.05, Figure 1B).

Comparison of Blood Vessel Density in NPDR Groups of Different AL

NPDR patients were divided into three groups according to AL, and VLD and VPD of the central region (1 mm), the inner ring region (1–3 mm), the outer ring region (3–6 mm) and the whole macular region (6 mm) were measured. Corresponding to the above four ranges, VLD of Group A was 7.9 \pm 3.0 mm⁻¹, 17.2 \pm 1.4 mm⁻¹, 17.9 \pm 1.1 mm⁻¹, 17.4 \pm 1.1 mm⁻¹, VLD of group B was 7.3 \pm 3.1 mm⁻¹, 16.5 \pm 1.4 mm⁻¹, 17.3 \pm 1.2 mm⁻¹, 16.9 \pm 1.2 mm⁻¹, VLD of group C was 6.0 \pm 2.6 mm⁻¹, 14.6 \pm 1.7 mm⁻¹, 14.2 \pm 1.8 mm⁻¹, 14.3 \pm 1.6 mm⁻¹. VLD of the inner loop area, the outer loop area and the overall VLD in the three groups A, B, and C was statistically different (*P*<0.05), while VLD in the central area was not statistically different in the three groups (*P*=0.244). VLD of the three groups A, B, and C were analyzed by multiple classification analysis in pairs. There was no statistically significant difference in VLD between groups A and B (*P*=0.518, 0.125, 0.944, 0.146). VLD of the inner ring region, outer ring region and the whole macular region of group C were compared with group A and group B, and the differences were statistically significant (*P*<0.05, Figure 2A).



Figure I Comparison of VLD (A) and VPD (B) between control group and group A. *P<0.05, **P<0.001. Abbreviations: VLD, vessel length density; VPD, vessel perfusion density.



Figure 2 Comparison of VLD (A) and VPD (B) in NPDR groups of different AL. *P<0.05, **P<0.001. Abbreviations: VLD, vessel length density; VPD, vessel perfusion density.

Corresponding to the above four regions, VPD of group A was 0.179 ± 0.072 , 0.415 ± 0.038 , 0.447 ± 0.026 , 0.432 ± 0.026 , VPD of group B was 0.162 ± 0.075 , 0.399 ± 0.037 , 0.432 ± 0.031 , 0.417 ± 0.029 , VPD of group C was 0.135 ± 0.063 , 0.350 ± 0.046 , 0.356 ± 0.043 , 0.352 ± 0.041 , respectively. VPD in the inner ring region, the outer ring region and the whole macular region had significant differences in the three groups A, B, and C (*P*<0.05), while VPD in the central region had no significant difference in the three groups (*P*=0.245). VPD of the three groups A, B, and C were compared in pairs by multiple analysis. There was no significant difference in VPD between groups A and B (*P*=0.453, 0.215, 0.152, 0.135). VPD in the inner ring region, outer ring region and the whole macular region in group C was compared with group A and group B, and the differences were statistically significant (*P*<0.05, Figure 2B).

Correlation Analysis of Blood Vessel Density and AL

There was a negative correlation between the inner ring region, outer ring region and the whole macular region of VLD and AL (r_s =-0.589, -0.690, -0.494; *P*<0.05), the same was true for VPD and AL (r_s =-0.553, -0.638, -0.614; *P*<0.05). However, VDL in the central region had no correlation with AL (*P*>0.05) (Table 2).

Comparison of SFCT and Parafoveal ORT Between Control Group and Group A

SFCT and parafoveal ORT of Group A were 230.1 \pm 52.9 µm, 144.0 \pm 12.2 µm, respectively, SFCT and parafoveal ORT of the control group were 270.7 \pm 68.0 µm, 141.1 \pm 10.1 µm, respectively. The difference in SFCT between group A and

		Axial Length (mm)		
		r _s	Р	
VLD (mm ⁻¹)	The central region	-0.254	0.066	
	The inner ring region	-0.589	<0.001	
	The outer ring region	-0.690	<0.001	
	The whole macular region	-0.696	<0.001	
VPD	The central region	-0.254	0.065	
	The inner ring region	-0.553	0.001	
	The outer ring region	-0.638	<0.001	
	The whole macular region	-0.614	<0.001	

 Table 2 Correlation Analysis Between Axial Length and Blood Vessel Density

Notes: Vessel length density (VLD) was defined as the total length of perfused vasculature per unit area in a region of measurement. Vessel perfusion density (VPD) was defined as the total area of perfused vasculature per unit area in a region of measurement. P<0.05 was considered significant.



Figure 3 Comparison of SFCT and parafoveal ORT between group A and the control group. **P*<0.05. **Abbreviations:** SFCT, subfoveal choroidal thickness; ORT, outer retina thickness.

control group was significant (P<0.05), but there was no significant difference in parafoveal ORT between group A and control group (P=0.432, Figure 3).

Comparison of SFCT and Parafoveal ORT Between NPDR Groups with Different AL

SFCT and parafoveal ORT of group A were 230.1 \pm 52.9 µm, 144.0 \pm 12.2 µm, respectively, SFCT and parafoveal ORT of group B were 190.4 \pm 50.0 µm, 139.2 \pm 12.4 µm, respectively, and SFCT and parafoveal ORT of group C were 111.4 \pm 52.1 µm, 130.0 \pm 6.6 µm, respectively. There were significant differences in SFCT and parafoveal ORT in groups A, B, and C (*P*<0.05). Furthermore, SFCT and parafoveal ORT of the three groups A, B, and C were compared by multiple analysis afterwards. The difference in SFCT between groups A, B, and C was significant (*P*=0.021, <0.001, <0.001). The difference in parafoveal ORT between groups A and C was significant (*P*<0.001) (Figure 4).

Correlation Analysis of SFCT and Parafoveal ORT with AL

There was a negative correlation between SFCT and AL (r_s =-0.594; P<0.001, Figure 5A), but there was no significant correlation between parafoveal ORT and AL (r_s =-0.152, P=0.278, Figure 5B).

Discussion

Capillary nonperfusion is an important feature of DR. VLD and VPD can be used as an indicator to observe the changes in DR capillary perfusion, and the changes in the macula vascular are sensitive indicators for the observation of DR.^{5,15} This study found that compared with the control healthy group, both VLD and VPD in NPDR patients decreased, suggesting that OCTA is a feasible and effective method to detect DR microvascular disease.⁶ Our results were consistent with previous report that retinal blood flow, and blood flow rate of diabetes mellitus (DM) patients were lower than those of normal people, and the diameter of blood vessels in the retinal blood vessels of patients with NDR and NPDR was narrower than normal people.¹⁶

DM not only causes retinal vascular changes but also affects choroidal vascular function.^{17,18} The change of SFCT is usually used to reflect the change of choroidal thickness in the macular area, and SFCT is greatly affected by the change of the axial length.¹⁹ In this study, we found significant differences in SFCT among the three groups of NPDRs with different AL, and there was a negative correlation between SFCT and AL. These results were in agreement with previous



Figure 4 Comparison of SFCT and parafoveal ORT in NPDR groups with different AL. *P<0.05, **P<0.001. Abbreviations: SFCT, subfoveal choroidal thickness; ORT, outer retina thickness.



Figure 5 The scatter plot of axial length with SFCT (**A**) and parafoveal ORT (**B**). Abbreviations: AL, axial length; SFCT, subfoveal choroidal thickness; ORT, outer retina thickness.

reports on the correlation between choroidal thickness and the axis of the eyes of different axes in non-diabetic people.^{20,21}

Studies have suggested that choroidal blood flow accounts for 85% of pulsatile ocular blood flow (POBF), and the change of POBF represents the change of choroidal blood flow.²² Al-Sheikh et al used OCTA to show that the blood flow of the choroid capillary layer in patients with high myopia was reduced compared with the control group.²³ Yang et al found that the POBF of patients with high myopia was significantly lower than that of the control group, and there was a negative correlation between POBF and axial length.²⁴ There was no significant difference between the parafoveal ORT between the NPDR group and the control group in this study, but the difference in parafoveal ORT among three different axis groups of NPDRs was significant, with a negative correlation between the parafoveal ORT thickness and AL. The

reason for the thinning of the outer retina in long axial eyes is believed to be mainly due to the axial extension and mechanical stretching of the retina.

With the improvement of OCTA, the retinal vessel density and foveal avascular area of different stages of DR have been quantified.²⁰ However, few studies focus on patients with long ocular axis when quantifying DR retinal blood vessels. The long eye axis is generally considered to be a protective factor for DR. Recently, Kim et al performed a retrospective study of DM patients with axial anisometropia with different axis and found that eyes with a longer axial axis had significantly lower DR lesions than those with a shorter axial axis.²⁵

In this study, we analyzed patients with NPDR with different axis, and found that macular density such as VLD and VPD, SFCT, and parafoveal ORT decreased with the increase of the axis. In an eye with a longer axial length, the retina has a larger surface area, which may explain the decrease of vessel density. Interestingly, a recent study explored the potential of wide-field swept-source OCTA (WF SS-OCTA) for the diagnosis and management of DR because the updated DR staging system has the ability to correlate detailed vascular and structural pathology over time with longitudinal imaging.²⁶ The development of this system may help monitor axial length and its effect on perfusion metrics.

Notably, this study has some limitations. First, this was a single-center cross-sectional study. Second, the sample size was relatively small and only included NPDR patients.

In conclusion, this study demonstrated that macular microvascular density and SFCT of NPDR patients with normal AL were lower than those of control group. In NPDR patients with different AL, macular microvascular density, SFCT, and parafoveal ORT decreased with the increase of AL.

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Disclosure

The authors report no conflicts of interest for this work.

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