Quantitative Analysis of the RPC Vessel Density and the RNFL Thickness in Patients with Type 2 Diabetes Mellitus by Using OCT Angiography

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Keywords

Diabetic retinopathy · Optical coherence tomography angiography · Optic disc · Radial peripapillary capillaries · Vessel density · Retinal nerve fibre layer

Abstract

Introduction: This study aims to compare the structural differences in the optic disc blood perfusion and the peripapillary retinal nerve fibre layer (pRNFL) thickness in age-matched healthy subjects and patients with type 2 diabetes mellitus (DM) by using quantitative analysis with optical coherence tomography angiography (OCTA). **Methods:** A cross-sectional cohort study on patients with type 2 DM with or without diabetic retinopathy (DR) and healthy subjects was conducted. The 4.5-mm scanning angio-disc pattern of the OCTA system was used to assess the optic disc. The analysed indices included radial peripapillary capillary (RPC) vessel density and pRNFL thickness. **Results:** A total of 78 eyes from 78 patients with type 2 DM, including 27 without clinical DR (NDR), 26 with non-proliferative DR (NPDR), and 25 with proliferative DR (PDR), and 28 age-matched healthy subjects were enrolled. The average RPC vessel density of the whole ($p < 0.001$) and the peripapillary ($p < 0.001$) regions was significantly different in different groups, whereas the pRNFL was not statistically significant ($p = 0.764$). Compared with that in healthy subjects, the RPC vessel densities in 4, 5, and 8 peripapillary sectors in NDR (all $p < 0.05$), NPDR

(all $p < 0.05$), and PDR (all $p < 0.05$) groups, respectively, were reduced. Compared with that in healthy subjects, the pRNFL thickness significantly decreased in the inferior nasal sector ($p = 0.001$) in NDR but significantly increased in the 2 sectors (all $p < 0.01$) in PDR. The DR severity was negatively correlated with the peripapillary RPC vessel density ($r = -0.583$, $p < 0.001$) but had no correlation with the pRNFL thickness ($r = -0.045$, $p = 0.648$). The positive correlation between the peripapillary RPC vessel density and the pRNFL thickness was statistically significant in the control ($r = 0.531$, $p = 0.004$), NDR ($r = 0.528$, $p = 0.004$), and NPDR ($r = 0.405$, $p = 0.040$) groups but not in the PDR group ($r = 0.394$, $p = 0.05$). **Conclusions:** The peripapillary RPC perfusion decreased with DR aggravation, which may be considered as a useful indicator of DR severity. However, the pRNFL thickness had little diagnostic power in differentiating healthy and DM eyes.

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Introduction

The prevalence of diabetes mellitus (DM) continues to rise rapidly and globally. The data from the International Diabetes Federation predict that 578 million people worldwide will have DM over the next decade [1, 2]. As one of the common chronic microvascular complications, diabetic retinopathy (DR) occurs in more than one-third of patients with DM and is the leading cause of blindness in the elder-

ly [3–5]. The pathophysiology of DR generally results from the thickening of the basement membrane, pericyte loss, and endothelial cell apoptosis, which may lead to microaneurysm formation and capillary occlusion and increase the vascular permeability by some ischaemia-activated growth factors [6, 7]. Mitochondrial damage and oxidative stress are associated with retinal neurodegeneration [8, 9]. DR is a microvascular disease and a neurodegenerative disease [10]. Many studies indicate that neuroretinal structural changes appear before evident vascular involvement [11]. Improvements in predicting the occurrence and monitoring the progress of DR are urgently needed due to insidious onset [12–14]. Fluorescein angiography enables the visualization of retinal circulation but has potential risks of intravenous contrast agents and difficulty in imaging the radial peripapillary capillary (RPC) region [15–17]. The optical coherence tomography angiography (OCTA) quantitatively detects perfusion volume through the high-resolution identification of moving red blood cells. The early identification of microcirculation disturbance and neurodegeneration by using OCTA is important. Most studies on DR have paid attention to the macular area in the past, whereas the focus on the optic disc is limited [18–20]. Interestingly, the axon density in the optic papilla is higher than that in the macula [21]. Thus, the retinal nerve fibre layer (RNFL) of the optic disc may be important in studying the neurodegeneration of DR. RPC is clinically and histologically confirmed to play a critically pivotal role in the arcuate fibres of RNFL [22, 23]. Therefore, the RPC vessel density in the optic disc may be a good assessment index. Previous studies reveal some quantitative indicators to describe the gradual changes in the peripapillary RPC vessel density and the pRNFL thickness [17, 24, 25]. However, few studies have included 8 sections of the peripapillary RPC vessel density in patients with DR at all stages. The optic nerve head can be comprehensively evaluated by analysing the peripapillary RPC vessel density and the pRNFL thickness in 8 sections. Similarly, by including all stages of DR, the development of DR can be fully clarified. The OCTA is used to quantitatively detect the structural changes in the RPC vessel density and the pRNFL thickness of the optic disc in patients with DM and evaluate its use in predicting the occurrence of DR.

Materials and Methods

Subjects

This prospective cross-sectional study adhered to the guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Affiliated Hospital of Weifang Medical

University (Approval No. 2019-010). The subjects were recruited from our department between March 2019 and January 2020, and subjects who met the criteria were consecutively enrolled.

Informed consent was obtained from all subjects to be included in our study. Healthy persons undergoing physical examinations were chosen as the control group. From these subjects, only those who met the inclusion (except DM diagnosis) and the exclusion criteria were included.

The inclusion criteria were as follows: diagnosis of type 2 DM, clear refractive media, ametropia <3 dioptres, and intraocular pressure ≤ 21 mm Hg. The exclusion criteria were as follows: presence of abnormal ocular circulation disease (such as glaucoma, optic neuropathy, macular oedema, and retinal vessel occlusion), history of any treatment for DR, hypertension, allergic constitution, or cardio-cerebrovascular disease.

Ophthalmic Examination

Each subject underwent comprehensive ophthalmic examinations, which included intraocular pressure, slit lamp microscopy, and ophthalmoscopy. Furthermore, the same senior ophthalmologist operated the colour fundus photography (Cidnary Medical Ltd., Wuhan, China) and OCTA (Optovue Inc., Fremont, CA, USA) after mydriasis. The eyes of the patients were divided into 3 groups, in accordance with the International Clinical Diabetic Retinopathy Disease Severity Scale [26], based on mydriatic fundus examination: (1) no abnormalities (DM with no DR, NDR); (2) microaneurysms, intraretinal haemorrhages, venous beading, intraretinal microvascular abnormalities, soft exudates, or hard exudates (non-proliferative DR, NPDR); and (3) neovascularization and vitreous/preretinal haemorrhage (proliferative DR, PDR). If both eyes were at the same stage, the severe eye was selected.

OCTA Data Measurements

The angio-disc 4.5-mm scan mode of the OCTA device with an 840-nm light source was chosen to capture a 4.5 mm $\times$ 4.5 mm area centred on the optic disc. The average vessel density over the entire range of measurements is defined as the whole VD. The software automatically obtained and segmented the optic disc images through an automatic fitting scan. The scope of the optic disc is automatically determined by the system, and the range within the boundary of the optic disc is defined as an inside disc. The peripapillary region was defined as a 750- μ m wide annulus extending from the optic disc boundary and divided into 8 sectors, namely, nasal superior (NS), nasal inferior (NI), inferior nasal (IN), inferior tempo (IT), tempo superior (TS), tempo inferior (TI), superior nasal (SN), and superior tempo (ST). The RPC vascular density and the NFL thickness of each segment were analysed. Each eye was measured thrice. Images with scanning quality <5 were excluded, and the highest-quality image of the subject was selected for preservation.

Statistical Analysis

Multivariate statistical analysis was completed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). The qualitative data of gender and laterality were expressed using frequency, and the χ^2 test was used to evaluate significant differences between groups. The Shapiro-Wilk test was used to evaluate the normality of quantitative data distribution. The quantitative data were presented as mean values $\pm$ standard deviation or median (interquartile range). The differences between groups were evaluated using analysis of variance and the Kruskal-Wallis test with Bonferroni correction. The

Table 1. Clinical and demographic characteristics of the study population

	Control	NDR	NPDR	PDR	<i>p</i> value
Patients, <i>n</i>	28	27	26	25	NA
Age, mean±SD, years	52.93±13.32	47.93±13.50	53.92±9.83	54.40±7.08	<i>F</i> = 1.624, <i>p</i> = 0.194
Gender, male/female	14/14	11/16	18/8	13/12	χ^2 = 4.487, <i>p</i> = 0.213
Laterality, right/left	15/13	10/17	14/12	14/11	χ^2 = 2.472, <i>p</i> = 0.480
DM duration, median (IQR), years	–	2.0 (0.3, 8.0)	8.5 (3.0, 15.5)	10.0 (7.0, 12.5)	<i>H</i> = 69.972, <i>p</i> < 0.001*

NA, not applicable; χ^2 , χ^2 test; *F*, Welch test; *H*, Dunnett's *t* test; DM, diabetes mellitus. * Statistical differences.

Fig. 1. Representative OCTA images of samples showing the vessel densities and the pRNFL thickness in the optic disc of each group (control, NDR, NPDR, and PDR). Optic disc neurovascular map measured through a 4.5 mm × 4.5 mm scan by using OCTA. **a** Radial peripapillary capillary layer is a 750-μm-wide annulus extending from the optic disc boundary and divided into 8 sectors. **b** En face OCTA of the RNFL thickness in the peripapillary region. OCTA, optical coherence tomography angiography; RNFL, retinal nerve fibre layer.

correlation coefficient was analysed using the Spearman's rank correlation. Results with a *p* value <0.05 were considered statistically significant.

Results

Population Characteristics
A total of 28 eyes from 28 control individuals and 78 eyes from patients with type 2 DM were eligible for the study. The 78 eyes of the patients with DM were divided

into 3 groups on the basis of the DR staging. The NDR, NPDR, and PDR groups contained 27, 26, and 25 eyes. As shown in Table 1, no statistical difference was observed in age, gender, and laterality within each group (*p* > 0.05). The duration of DM among the 3 DM groups was comparable (*p* < 0.001).

OCTA Measurements of the Optic Disc
Figure 1 shows the representative OCTA image samples of the vessel densities and the pRNFL thickness in the optic disc. The RPC vessel density in the whole region,

Table 2. Representative RPC vessel density and RNFL thickness for the normal and diabetic groups

	Control	NDR	NPDR	PDR	F/H value	<i>p</i> value
RPC, mean±SD, %						
Whole	50.61±1.92	48.67±1.96	47.62±1.83	46.08±3.41	<i>F</i> = 16.761	<0.001*
Inside disc	48.88±4.92	49.87±4.53	44.79±6.85	46.92±6.40	<i>F</i> = 4.007	0.009*
Peripapillary	53.83±2.67	50.84±2.12	50.47±1.89	48.15±3.92	<i>F</i> = 19.204	<0.001*
RNFL, median (IQR), μm						
Peripapillary	116.00 (110.50, 120.75)	113.00 (102.00, 121.00)	109.00 (101.00, 127.50)	115.00 (96.00, 137.00)	<i>H</i> = 1.154	0.764 ^a

RNFL, retinal nerve fibre layer; RPC, radial peripapillary capillary. * Statistically significant analysis of variance. ^a Spearman correlation coefficient test.

Fig. 2. Column of VD and RNFL thickness in the optic disc of each group (control, NDR, NPDR, and PDR). The means (bar) and standard deviations (whiskers) are illustrated in Table 2. Significant tests ($p < 0.05$) are marked with brackets in the figures. VD, vessel density; RNFL, retinal nerve fibre layer; RPC, radial peripapillary capillary.

inside disc, and peripapillary region and the pRNFL thickness of 4 groups are reported in Table 2 and Figure 2. The one-way analysis of variance showed that the average RPC vessel density in the whole region of the NDR ($48.67\% \pm 1.96\%$), NPDR ($47.62\% \pm 1.83\%$), and PDR ($46.08\% \pm 3.41\%$) groups was significantly lower than that of the control group ($50.61\% \pm 1.92\%$, $p < 0.001$). The average

RPC vessel densities of the inside disc region were $48.88\% \pm 4.92\%$, $49.87\% \pm 4.53\%$, $44.79\% \pm 6.85\%$, and $46.92\% \pm 6.40\%$ in the control, NDR, NPDR, and PDR groups, respectively, and these values were significantly different ($p = 0.009$). The average RPC vessel density in the peripapillary region of the NDR ($50.84\% \pm 2.12\%$), NPDR ($50.47\% \pm 1.89\%$), and PDR ($48.15\% \pm 3.92\%$) groups was also sig-

nificantly lower than those of the control group ($53.83\% \pm 2.67\%$, $p < 0.001$). The Kruskal-Wallis test showed that the pRNFL thickness had no significant difference amongst the groups ($p = 0.764$, Table 2). However, the pRNFL thickness showed a trend of decreasing first and then increasing (Fig. 2d). On a subgroup analysis using a multiple-comparison test based on the stage of the DR, the average RPC vessel density in the whole region between the control and the NDR ($p = 0.003$), the control and the NPDR ($p < 0.001$), the control and the PDR ($p < 0.001$), and the NDR and the PDR ($p = 0.012$) groups showed significant differences (Fig. 2a), and the average RPC vessel density in the peripapillary region between the control and the NDR ($p < 0.001$), the control and the NPDR ($p < 0.001$), the control and the PDR ($p < 0.001$), the NDR and the PDR ($p = 0.001$), and the NPDR and the PDR ($p = 0.003$) showed significant differences, whereas the other comparisons showed no significant difference (Fig. 2c). The inside disc RPC vessel density in the NPDR group was significantly decreased compared with those in the control ($p = 0.01$) and the NDR ($p = 0.002$) groups (Fig. 2b). Table 3 displays the peripapillary region divided into 8 sections. Compared with the control group, the PDR, NPDR, and NDR groups had significantly reduced vessel density in 8, 5 (i.e., NS, NI, IN, IT, and TS), and 4 (i.e., NS, NI, IN, and IT) subregions, respectively (all $p < 0.05$). The pRNFL thickness of the NDR group was significantly reduced in the IN subregion compared with that of the control group ($p = 0.001$), whereas the pRNFL thickness of the PDR group was significantly increased in the TS and the TI subregions compared with that of the control group (all $p < 0.01$).

Correlations amongst RPC Vessel Density, pRNFL Thickness, and DR Severity

The average RPC vessel density in the whole ($r = -0.590$, $p < 0.001$) and the peripapillary ($r = -0.583$, $p < 0.001$) regions was significantly correlated with DM severity. However, no statistically significant association was found between the inside disc RPC vessel density and the DR severity ($r = -0.191$, $p = 0.050$) and between the pRNFL thickness and the DR severity ($r = -0.045$, $p = 0.648$; Table 4). The results of Spearman correlation analysis between the RPC vessel density and the pRNFL thickness in the peripapillary region amongst different groups are shown in Figure 3. The correlation between the peripapillary RPC vascular density and the pRNFL thickness in the PDR group was not statistically significant ($r = 0.394$, $p = 0.05$), whereas that in the other groups was positively correlated.

Table 3. Specific subregions of RPC vessel density and pRNFL thickness of the normal and the diabetic groups

Sector	Control	NDR (control vs. NDR, p value)	NPDR (control vs. NPDR, p value)	PDR (control vs. PDR, p value)
Peripapillary capillary density, %				
Nasal superior	51.00 (49.00, 53.00)	46.00 (45.00, 49.00) ($p = 0.004$)	47.50 (45.75, 50.00) ($p = 0.037$)	45.00 (41.00, 47.50) ($p < 0.001$)
Nasal inferior	49.50 (45.25, 52.75)	46.00 (42.00, 48.00) ($p = 0.004$)	46.00 (43.75, 48.00) ($p = 0.009$)	43.00 (39.00, 48.50) ($p = 0.002$)
Inferior nasal	53.50 (50.00, 55.75)	49.00 (47.00, 52.00) ($p = 0.004$)	49.00 (45.00, 51.75) ($p = 0.002$)	48.00 (42.50, 50.00) ($p < 0.001$)
Inferior tempo	60.00 (57.00, 63.00)	56.00 (54.00, 58.00) ($p = 0.023$)	56.00 (53.75, 59.00) ($p = 0.018$)	56.00 (50.50, 58.00) ($p = 0.001$)
Tempo superior	58.00 (53.50, 60.00)	56.00 (52.00, 58.00) ($p = 0.192$)	54.00 (50.00, 57.25) ($p = 0.017$)	53.00 (48.00, 57.00) ($p = 0.002$)
Tempo inferior	54.00 (51.25, 56.00)	52.00 (50.00, 55.00) ($p = 1.000$)	53.00 (48.00, 56.00) ($p = 1.000$)	50.00 (45.00, 53.00) ($p = 0.035$)
Superior nasal	52.50 (50.00, 54.75)	51.00 (48.00, 54.00) ($p = 0.607$)	50.50 (46.75, 53.00) ($p = 0.126$)	49.00 (43.50, 52.50) ($p = 0.009$)
Superior tempo	57.50 (54.25, 60.00)	56.00 (53.00, 58.00) ($p = 0.338$)	55.00 (51.00, 58.00) ($p = 0.129$)	52.00 (50.00, 57.00) ($p = 0.002$)
Peripapillary RNFL thickness, μm				
Nasal superior	108.50 (100.25, 122.00)	100.00 (94.00, 117.00) ($p = 0.269$)	97.50 (91.25, 116.25) ($p = 0.269$)	104.00 (79.50, 121.00) ($p = 0.269$)
Nasal inferior	90.50 (78.25, 97.75)	83.00 (73.00, 99.00) ($p = 0.664$)	81.00 (74.75, 105.00) ($p = 0.664$)	78.00 (59.00, 113.00) ($p = 0.664$)
Inferior nasal	156.50 (140.25, 163.75)	130.00 (121.00, 148.00) ($p = 0.001$)	132.00 (99.50, 168.00) ($p = 0.533$)	128.00 (105.50, 170.50) ($p = 0.115$)
Inferior tempo	153.50 (139.25, 162.00)	146.00 (132.00, 175.00) ($p = 0.906$)	144.00 (135.75, 168.25) ($p = 0.906$)	161.00 (123.00, 175.00) ($p = 0.906$)
Tempo superior	80.00 (74.25, 85.00)	85.00 (76.00, 95.00) ($p = 0.857$)	83.50 (74.75, 92.50) ($p = 1.000$)	96.00 (81.00, 112.00) ($p = 0.003$)
Tempo inferior	74.00 (67.50, 79.75)	77.00 (70.00, 86.00) ($p = 1.000$)	81.00 (70.75, 91.50) ($p = 0.448$)	91.00 (77.50, 111.50) ($p = 0.007$)
Superior nasal	141.50 (132.25, 163.25)	133.00 (124.00, 151.00) ($p = 0.389$)	142.50 (127.75, 155.00) ($p = 0.389$)	137.00 (108.00, 167.50) ($p = 0.389$)
Superior tempo	135.00 (116.00, 150.00)	138.00 (122.00, 160.00) ($p = 0.577$)	144.00 (112.00, 151.25) ($p = 0.749$)	146.00 (112.50, 175.00) ($p = 0.143$)
p values are compared to the corresponding control subjects. RNFL, retinal nerve fibre layer; RPC, radial peripapillary capillary.

Fig. 3. Scatter plots of the linear relationships between RPC VD and RNFL thickness of the peripapillary region in the control (a), NDR (b), NPDR (c), and PDR (d) groups. VD, vessel density; RNFL, retinal nerve fibre layer; RPC, radial peripapillary capillary.

Table 4. The relationship between RPC vessel density or pRNFL thickness and DR severity

DR severity	RPC vessel density			pRNFL thickness
	whole	inside disc	peripapillary	
<i>r</i>	−0.590	−0.191	−0.583	−0.045
<i>p</i> value	<0.001**	0.050	<0.001**	0.648

Spearman's rank correlation coefficient was utilized to examine relationships. RNFL, retinal nerve fibre layer; RPC, radial peripapillary capillary; DR, diabetic retinopathy. ** $p < 0.01$.

Discussion

This study reveals the changes in the RPC vessel density and the pRNFL thickness at different stages of DR. The quantitative method used in previous studies on DR has analysed the macular perfusion parameters, such as perfusion density, foveal avascular zone area, and ganglion cell complex thickness [19, 27–29]. Many studies have confirmed that the progression of DM is accompanied with the aggravation of macular ischaemia. However, few studies attempt to scan the optic disc. It has been shown that the peripapillary region has an earlier microvascular susceptibility than the macula [30]. Here, we aim

to provide complete data and deep insight into the progressive changes in DR in the optic disc.

The central retinal artery is responsible for the blood supply of the RNFL, and the posterior ciliary artery and few branches of the central retinal artery supply the deeper layer of the optic disc, which is in the vicinity of the sieve plate area [31]. Slender RPC vessels are the superficial capillary network of RNFL, which easily form anastomosis with the surrounding blood vessels. Some researchers have found a positive correlation between RPC and RNFL in healthy persons, which is also found in our study [32, 33]. Vujosevic et al. [30] found a significant positive association between them not only in healthy individuals but also in diabetic patients without DR, which was also found in our study. Thus, RPC is closely linked to RNFL, and RPC may be responsible for RNFL nourishment [22, 34]. Furthermore, we found no correlation between RPC and RNFL in patients with PDR, however possibly due to the pseudo-thickening of the nerve fibre layer caused by retinal oedema. Our study shows that compared with healthy subjects, the patients in the NDR, NPDR, and PDR groups have reduced RPC vessel density in 4, 5, and 8 peripapillary sectors. Therefore, microvascular changes may be an early and reliable marker for preclinical DR. This result is similar to that in previous studies. However, some inadequacies exist in their studies. For instance, Cao et al. [24] have not included the NPDR and the PDR groups, and Li et al. [25] have not incorporated the PDR group. Both researchers cannot determine the progressive variation in the OCTA parameters with DR aggravation. Our study has confirmed a negative correlation between peripapillary RPC and DR aggravation. DR is primarily characterized by capillary reorganization and degeneration [35]. Several mechanisms may explain the reduced RPC vessel density. First, hyperglycaemia leads to the destruction of the capillary structure, which is manifested by the thickening of the basement membrane and loss of pericytes, damaging the integrity of the vascular endothelium [36]. In turn, the leaked fluid presses the blood vessels to stenosis or even occlusion, resulting in vessel density reduction [37]. Second, retinal perfusion is affected by the reduced erythrocyte deformability and elevated blood viscosity in patients with DM [38]. In addition, the high energy requirements of RNFL's unmyelinated nerve fibres make RPC extremely sensitive to ischaemic injury [32]. Thus, poor RPC regulation may also be one of the reasons for such a situation [39].

Interestingly, we found there was no significant trend in vessel density of the inside disc region between the 4

groups. First, the vessel density of the NPDR group had a statistically significant reduction both in comparison with the control group and the NDR group, which is consistent with the mechanism of the RPC vessel density reduction mentioned above. Second, the comparisons between other groups were not found to be statistically different. We speculate that the trend of increasing RPC vessel density at the PDR stage may be due to the presence of slender neovascularization on the disc, which is difficult to detect and inevitably introduced errors; the increased trend of RPC vessel density during the NDR stage may be due to the more crowded anatomy of the peripapillary region compared with the neurovascular distribution in other regions of the retina, which makes it easier to generate compensatory mechanism due to ischaemia. These speculations, however, await further validation.

Furthermore, the average pRNFL thickness tends to decrease in patients with NDR and NPDR. These results are consistent with the recently published studies [25, 40]. Through 8 partitions of pRNFL, we have found that the pRNFL thickness in NDR is considerably decreased only in inferior nasal sectors than that in healthy subjects. Differences in the peripapillary RPC vessel density are also concentrated in the inferior and the nasal regions, which may explain the trophic effect of RPC on RNFL [32]. Satue et al. [21] believe that the main difference in pRNFL thickness between the patients without DR and the healthy subjects is concentrated in the inferior quadrant, and Shin et al. [17] have confirmed that the neurodegenerative changes caused by DM primarily affect the inferior quadrant of pRNFL. Vujosevic et al. [30] found an early reduction of RNFL in the inferior quadrant even in diabetic patients without DR. Their studies have great similarity with our study. However, these studies use optical coherence tomography to measure the thickness of pRNFL. Differences in partitioning may be the reason for the nuances.

Notably, our study has found no correlation between pRNFL thickness and the aggravation of DR. This finding is consistent with the recently published study [40]. The reasonable explanation for this manifestation is that the RPC perfusion reduction at the early stage results in pRNFL nutritional impairment and progressive loss of ganglion and glial cells, and fluid leakage and tissue oedema at the late stage lead to pRNFL pseudo-thickening [41]. This phenomenon is also the reason for the absence of correlation between the peripapillary RPC vessel density and the pRNFL thickness in patients with PDR, but negative correlations are found between patients with DM at varying diabetic stages and healthy subjects. Thus, judg-

ing the severity of DR by evaluating pRNFL thickness may not be advisable.

Interestingly, diabetics at any stage show significant changes in most areas of the peripapillary RPC than in those in pRNFL compared with the healthy subjects. This finding suggests that OCTA microvascular changes are more sensitive and extensive than neural structural changes and that the changes in the RPC vessel density and the pRNFL thickness do not occur simultaneously, but the cause and effect relationship between them is uncertain.

Limitations exist in this study. First, the OCTA selects a relatively small scan range. Research shows that the RPC in the arcuate fibre regions still exists in the 5.5-mm range, which extends outward from the optic disc [22, 42]. However, the clarity of the blood flow signal decreases with increasing scanning range. Second, further studies based on longitudinal data rather than cross-sectional data should be made, which can be used to investigate the causal relationship between the reduced peripapillary perfusion and the reduced pRNFL thickness. Third, whether DM medication therapy affects retinal parameters is unknown, which may be one of the reasons for the different conclusions. Last, we have ruled out patients with diabetic macular oedema, which affects the comprehensive understanding of the neurovascular changes in the optic disc of DR. However, with these criteria, we have excluded the factors affecting pRNFL thickness due to macular oedema.

In general, we think that the vascular indicator of the peripapillary RPC perfusion decreases with the aggravation of the disease, which occurs in preclinical DR, but the sensitivity of the pRNFL thickness as a morphological indicator is lower than that as a vascular indicator. Currently, the treatment of DR is limited, applies principally to the advanced stage, and has short-term efficacy and side effects [43]. Early screening should be the mainstay

of DR to achieve awareness of the preclinical disease and delay the progression. As a non-invasive and rapid detection method, the OCTA can quantify blood perfusion and morphological changes in the retina of patients with pre-clinical DR.

Statement of Ethics

This protocol was guided by the Declaration of Helsinki and was approved by the Research Ethics Committee of the Affiliated Hospital of Weifang Medical University (Approval No. 2019-010). Informed, written consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Fan Jia designed experiments; Yan Li and Na Li directed, reviewed, and checked the experiments; Fan Jia, Min Zhang, Chunyuan Song, and Jin Yang carried out experiments; Fan Jia and Min Zhang analysed experimental results; Min Zhang wrote the manuscript and drew the statistical charts; Yan Li and Shuna Wang modified the language; Shuna Wang, Kaili Yang, and Na Li assisted in data collection.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

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