Article

Peripapillary Region Perfusion and Retinal Nerve Fiber Layer Thickness Abnormalities in Diabetic Retinopathy Assessed by OCT Angiography

Lun Liu[1], Yong Wang[1], Hua Xing Liu[1], and Jian Gao[1]

[1] Department of Ophthalmology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

Correspondence: Jian Gao, Department of Ophthalmology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China. e-mail: shuijinglovegj@126.com

Purpose: To quantify peripapillary region perfusion and retinal nerve fiber layer (RNFL) thickness abnormalities in different stages of diabetic retinopathy (DR) using optical coherence tomography angiography (OCTA).

Methods: Seventy-two eyes of 72 patients with diabetes were included as follows: 23 with no DR (No DR), 24 with mild-to-moderate nonproliferative DR (mild DR), 25 with severe nonproliferative to proliferative DR (severe DR), and 26 age-matched healthy controls. All eyes underwent a 4.5 × 4.5-mm rectangle scan centered on the optic nerve head. Vessel densities and RNFL thickness for the peripapillary area were calculated.

Results: A statistically significant decrease in vessel density was found in the peripapillary region with increased DR severity (all $P < 0.001$). There were significant correlations between DR severity and vessel density in the peripapillary region ($P < 0.001$), but not between DR severity and RNFL thickness ($P > 0.05$). There was a significantly positive correlation between vessel density and RNFL thickness of the peripapillary region in the mild DR group ($r = 0.726$, $P < 0.001$) but not in the no DR group ($r = 0.008$, $P = 0.973$) or the severe DR group ($r = 0.281$, $P = 0.173$).

Conclusions: Vessel density in the peripapillary region correlated significantly with DR severity, decreasing with DR aggravation. There was no obvious correlation observed between RNFL thickness and DR severity.

Translational Relevance: Vessel density in the peripapillary region, assessed by OCTA technology, can be potentially useful for analyzing and monitoring retinal nerve changes in DR patients.

Introduction

Diabetic retinopathy (DR) is a leading cause of acquired visual impairment and is increasingly becoming one of the world's most significant public health challenges.[1,2] Numerous substantial advances have been made in understanding the disease during the past few decades.[3,4] However, the pathogenesis of DR remains unclear.

For many years, DR has been considered a type of microvascular problem. Recently, both neural and microvascular factors have been associated with DR.[5] Furthermore, retinal neurodegeneration has been found to have a significant role in the pathogenesis of DR, including apoptosis of retinal neuronal cells and peripapillary retinal nerve fiber layer (RNFL) thinning.[6] The RNFL is composed of retinal ganglion cell axons and makes up the innermost neural layer of the retina.[7] It has been proven that the nutritional demands of the RNFL are likely to be partially satisfied by radial peripapillary capillaries (RPCs).[8] Both histologic and clinical studies suggest that RPCs play an important role in the RNFL arcuate fiber area.[8,9] Many pathological changes, such as the Bjerrum scotoma, cotton wool spot, intraretinal hemorrhage, and ischemic optic neuropathy, have nerve fiber defects consistent with the distribution of RPCs.[10–12] Examining the changes of the RNFL and RPCs can provide an improved clinical understanding of neurodegeneration during the different stages of

DR. However, there is little quantitative information regarding RPC microcirculation in diabetic patients.

A recent noninvasive imaging technique, optical coherence tomography angiography (OCTA), shows repeatability and reproducibility in vessel density measurements of RPCs and thickness measurements of the RNFL.[13,14] In the present study, OCTA was used to quantitatively analyze the changes in the vessel density of RPCs and RNFL thickness in the optic nerve head of DR patients in different stages, and their correlation with DR severity.

Methods

Study and Patients

This cross-sectional study was performed at the First Affiliated Hospital of Anhui Medical University and followed the tenets of the Declaration of Helsinki. This study was approved by the institutional review board at the First Affiliated Hospital of Anhui Medical University.

Inclusion criteria were healthy eyes, eyes of patients with diabetes without DR, and eyes with DR, based on clinical assessment by retinal specialists. The exclusion criteria were eyes with opaque media that precluded fundus examination, glaucoma, a high refractive error (>6 diopters), uveitis, other retinal diseases, and ocular trauma. We also excluded eyes with evidence of optic disc neovascularization, optic disc edema, or those that had OCTA images with a scan quality index (SQI) of less than 5.

Ocular Examination

Each patient underwent a series of ocular examinations, including a biomicroscopy examination of the fundus, OCTA, color fundus photography, and intraocular pressure measurement using a noncontact tonometer. Each eye was graded using the Early Treatment Diabetic Retinopathy Study classification.[15] Based on the DR grade, which was determined by fundus photography and examinations, the less serious eye in one patient was chosen. When the two eyes of one patient had the same DR grade, the eye with a higher SQI was chosen. The eyes of the patients with diabetes were divided into the following three groups according to the DR grade: a diabetes mellitus without DR (no DR) group, a mild-to-moderate nonproliferative DR (mild DR) group, and a severe nonproliferative to proliferative DR (severe DR) group.

Optical Coherence Tomography Angiography Imaging and Image Processing

OCTA imaging of the optic disc was performed by using an AngioVue OCTA system (Optovue, Inc., Fremont, CA). A 4.5 × 4.5-mm rectangle scan centered on the optic nerve head was performed. The newly developed, built-in AngioAnalytics software (version 2017.1.0.151; Optovue, Inc.) was used to evaluate vessel density and RNFL thickness. The software defines the peripapillary region as a 1.0-mm wide round annulus extending from the optic disc boundary (Fig. 1A). The peripapillary vessels were analyzed in superficial retinal layers from the RPC segment that extends from the inner limiting membrane to the nerve fiber layer (Fig. 1B); peripapillary vessel density was defined as the percentage of the area occupied by the vessels in the peripapillary region. The software calculated the vessel density for the peripapillary area (Fig. 1C). Simultaneously, the average RNFL thickness for the peripapillary area was recorded (Fig. 1C). Color maps were also used to show the vessel density (Fig. 1D) and RNFL thickness (Fig. 1E) immediately. Error in automatic segmentation sometimes occurred; in these cases, we manually corrected the entire scan volume.

Statistical Analysis

SPSS software for Windows, version 21.0 (IBM Corp., Armonk, NY), was used for statistical analysis. Normality of data was assessed using the Shapiro-Wilk test. All data are shown as the mean ± standard deviation (SD), median and interquartile range (IQR, 25th–75th percentile), or percentages if appropriate. Differences in the data were assessed using the *t*-test or analysis of variance (ANOVA). Correlations between the OCTA parameters and DR severity were examined by using the Kendall's tau correlation coefficient; DR severity was defined as a continuous or categoric variable. A Spearman rank correlation analysis was performed to determine the relationships between vessel density and RNFL thickness. A P value <0.05 was considered statistically significant.

Results

Patient Characteristics

The study included 26 healthy, age-matched controls (26 eyes) and 72 patients with diabetes (72 eyes). The 72 eyes of patients with diabetes were
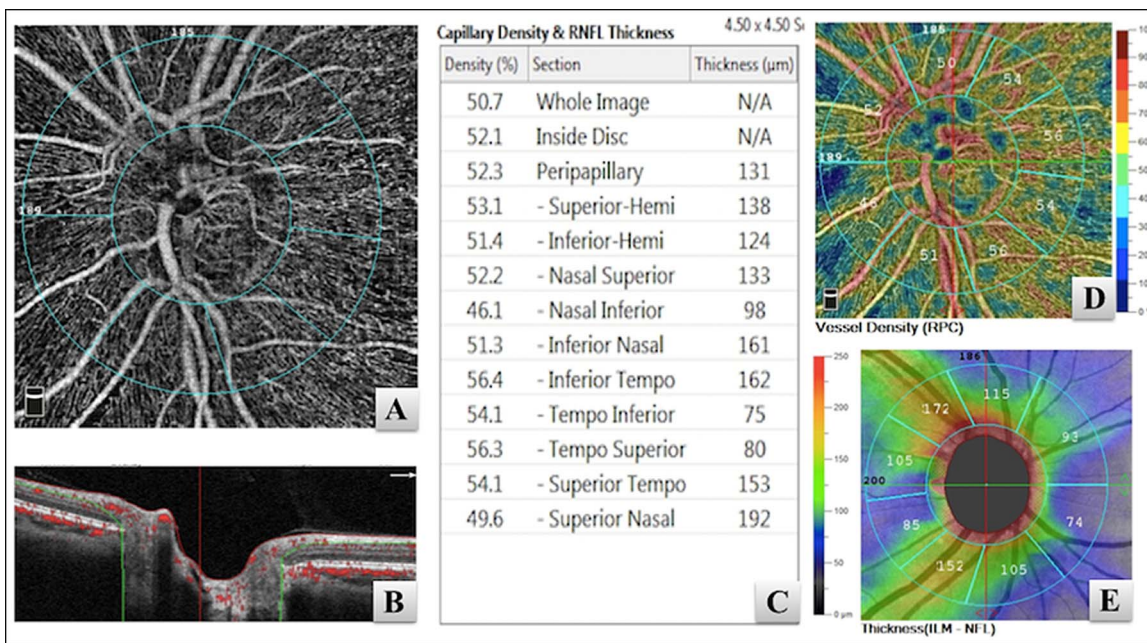
Figure 1. Measurement of OCTA parameters centered on the optic nerve head with a 4.5-mm² rectangle scan. (A) The software defines the peripapillary region as a 1.0-mm wide round annulus extending from the optic disc boundary. (B) Peripapillary vessels were analyzed in superficial retinal layers from the RPC segment, which extends from the inner limiting membrane to the nerve fiber layer. (C) Vessel densities and RNFL thickness for the peripapillary area. Color maps show the vessel density (D), and RNFL thickness (E) for the peripapillary region.

divided into the following three groups based on the DR grade: 23 eyes had no DR, 24 eyes had mild DR, and 25 eyes had severe DR. All demographic data, general clinical characteristics, and SQI values of the images are shown in Table 1.

Analysis of Vessel Density in the Peripapillary Region

Figure 2 shows a representative sample of vessel density in the peripapillary region with increasing DR severity. The average RPC vessel density for the peripapillary area is reported in Table 2 for each group. A statistically significant decrease in vessel density was found in the peripapillary region among the four groups ($P < 0.001$) (Table 2). Using the Kendall's tau correlation coefficient analysis, significant correlations between DR severity and vessel density in the peripapillary region were observed ($P < 0.001$) (Table 3). When post hoc multiple comparisons were performed, statistically significant decreases in the vessel density were observed in the mild severe DR groups when compared with the control group (Table 2, Fig. 3A). Statistically significant decreases in the

Table 1. Demographic and Clinical Characteristics of the Study Participants

	Control	No DR	Mild DR	Severe DR	P Value
Patients, n	26	23	24	25	
Eyes, n	26	23	24	25	
Sex					
Female, n	7	5	13	10	
Male, n	19	18	11	15	
Age, y, mean ± SD	51.88 ± 7.03	48.78 ± 11.79	50.79 ± 9.02	52.00 ± 9.33	0.615
Disease duration, median (IQR)	-	3 (1–8)	6 (5–11)	10 (6–12)	<0.001*
SQI, mean ± SD	7.54 ± 0.99	7.39 ± 0.99	7.25 ± 1.11	7.16 ± 1.03	0.581

* Indicates statistically significant difference between the groups. A value of $P < 0.05$ was considered statistically significant.
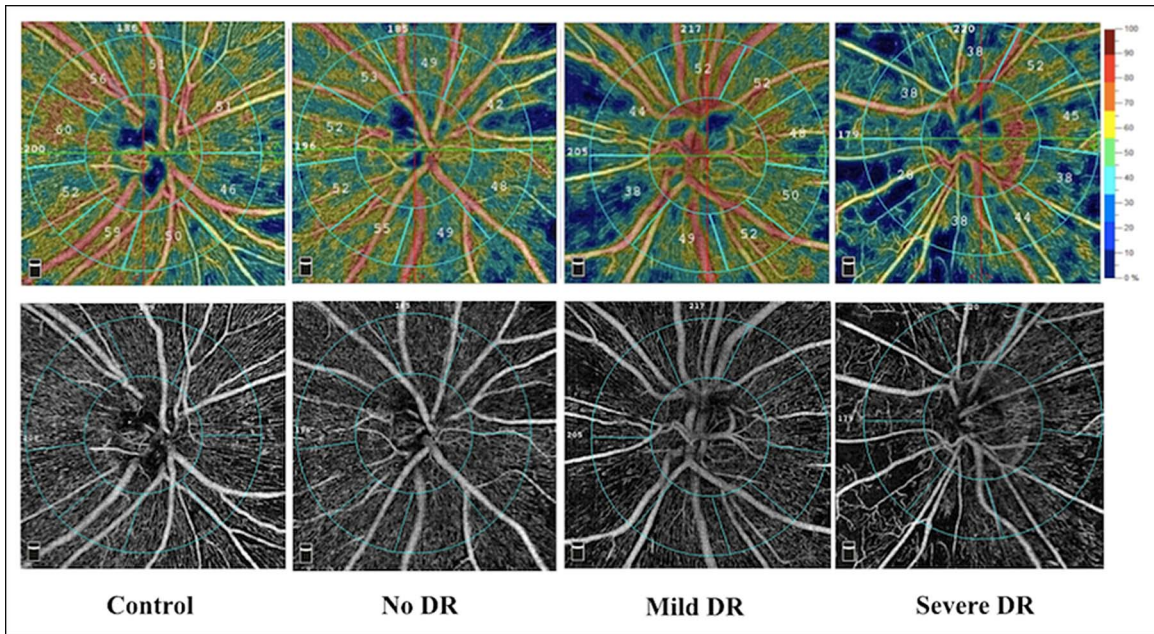
Figure 2. Representative sample of vessel densities in the peripapillary region for each group (control, no DR, mild DR, and severe DR). *Top row*: vessel densities in the peripapillary region shown by color maps. *Bottom row*: en face OCTA of the radial peripapillary capillaries in the peripapillary region.

vessel density were observed in the severe DR group when compared with the no DR group (Table 2, Fig. 3A). Furthermore, a statistically significant decrease in vessel density was observed in the severe DR group when compared with the mild DR group (Table 2, Fig. 3A). No significant difference in the vessel density was observed between the control group and the no DR group (Table 2, Fig. 3A).

Analysis of Retinal Nerve Fiber Layer Thickness in the Peripapillary Region

Figure 4 shows a representative sample of RNFL thickness in the peripapillary region with increasing DR severity. The average RNFL thicknesses in the peripapillary area are reported in Table 2. No significant difference in RNFL thickness was found in the peripapillary region between the 4 groups ($P >$ 0.05) (Table 2). Using Kendall's tau correlation coefficient analysis, no significant correlation between DR severity and RNFL thickness was found in the peripapillary region ($P > 0.05$) (Table 3). Using the student's t-test, statistically significant decreases in the RNFL thickness were observed in the no DR group when compared with the control group ($P < 0.05$) (Fig. 3B). No significant difference in RNFL thickness was found in the peripallary region between the no DR, mild DR, and severe DR groups (all $P >$ 0.05) (Fig. 3B).

Correlations Between Vessel Density and Retinal Nerve Fiber Layer Thickness With Increasing Diabetic Retinopathy Severity

The results of the Spearman correlation analysis of vessel density and RNFL thickness of the entire

Table 2. Comparisons of Vessel Density and RNFL Thickness in the Peripapillary Region Between the Four Study Groups

Peripapillary (mean ± SD)	Control	No DR	Mild DR	Severe DR	F Value	P Value
Vessel density, %	53.87 ± 2.17	52.64 ± 3.06	50.63 ± 5.42	46.15 ± 4.26	18.908	<0.001[a]
RNFL thickness, μm	119.50 ± 9.94	112.78 ± 9.98	113.04 ± 18.35	116.56 ± 21.94	0.992	0.4

[a] Multiple comparisons are tested using one-way ANOVA with a post-hoc test, control > mild DR ($P < 0.05$), Control > severe DR ($P < 0.001$), No DR > severe DR ($P < 0.001$), mild DR > severe DR ($P < 0.001$).
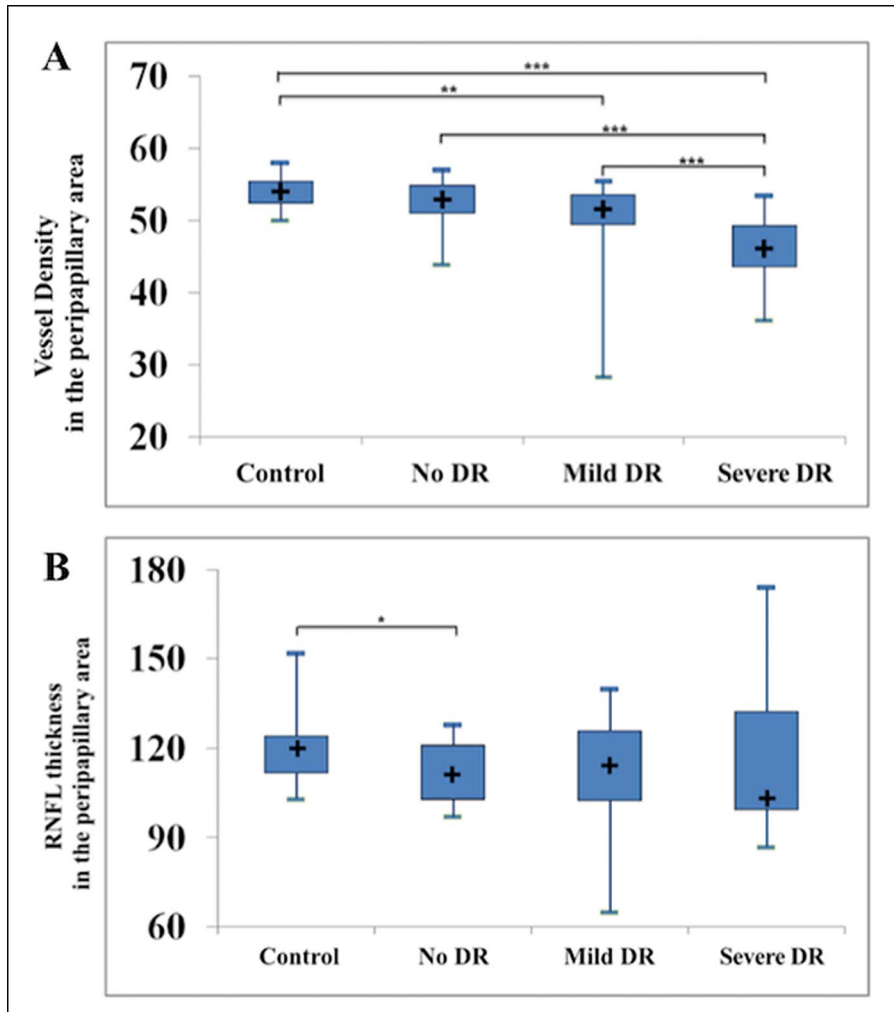
Figure 3. Boxplots of vessel density (A) and RNFL thickness (B) in the peripapillary region for each group (control, no DR, mild DR, and severe DR). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

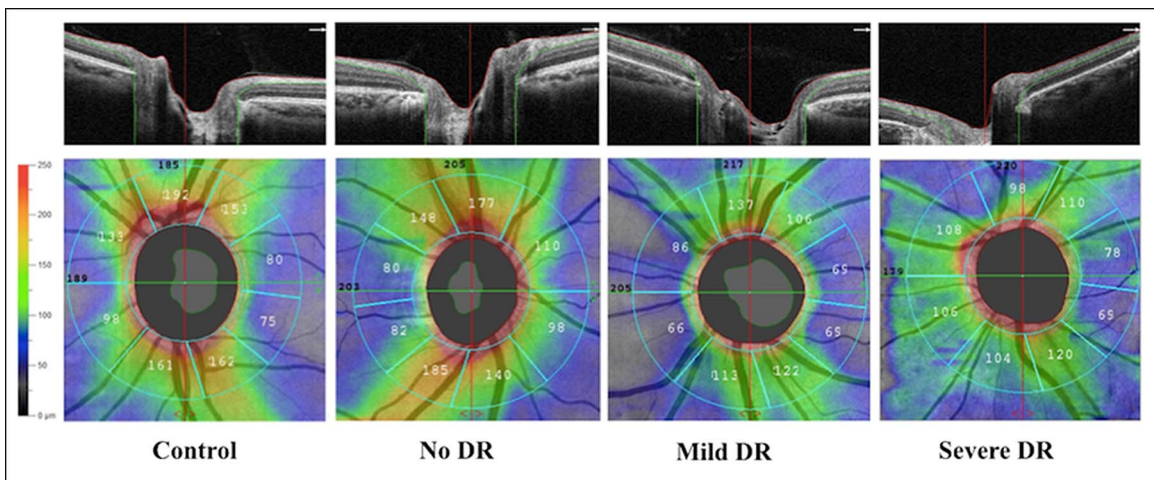**Figure 4.** Representative samples of RNFL thickness in the peripapillary region for each group (control, no DR, mild DR, and severe DR). *Top row*: B scan of the RNFL in the peripapillary region. *Bottom row*: RNFL thickness in the peripapillary region shown by color maps.

Table 3. Kendall's Tau Correlation Coefficient Analysis Showing Correlations Between OCTA Parameters of the Peripapillary Region and Severity of DR

Peripapillary (Mean ± SD)	Control	No DR	Mild DR	Severe DR	Correlation Cofficient	P Value
Vessel density, %	53.87 ± 2.17	52.64 ± 3.06	50.63 ± 5.42	46.15 ± 4.26	−0.520	<0.001*
RNFL thickness, μm	119.50 ± 9.94	112.78 ± 9.98	113.04 ± 18.35	116.56 ± 21.94	−0.074	0.334

* Indicates statistically significant difference between the groups. A value of $P < 0.05$ was considered statistically significant.

peripapillary region among the different groups are shown in Figures 5A through 5C. Generally, there was a significant positive correlation between vessel density and RNFL thickness of the entire peripapillary region in the mild DR group ($r = 0.726$, $P < 0.001$) (Fig. 5B). However, no statistically significant associations were found between vessel density and RNFL thickness of the peripapillary region in the no DR group ($r = 0.008$, $P = 0.973$) and the severe DR group ($r = 0.281$, $P = 0.173$) (Figs. 5A, 5C).

Discussion

In this study, we evaluated the correlation between microvascular and RNFL thickness changes in the peripapillary region and disease severity in eyes with DR via OCTA.

The RPCs are a unique capillary plexus within the inner aspect of the RNFL.[16,17] The high energy demands placed upon the nonmyelinated axons located in the RNFL make it highly vulnerable to injury from ischemic insults.[18,19] Previous studies
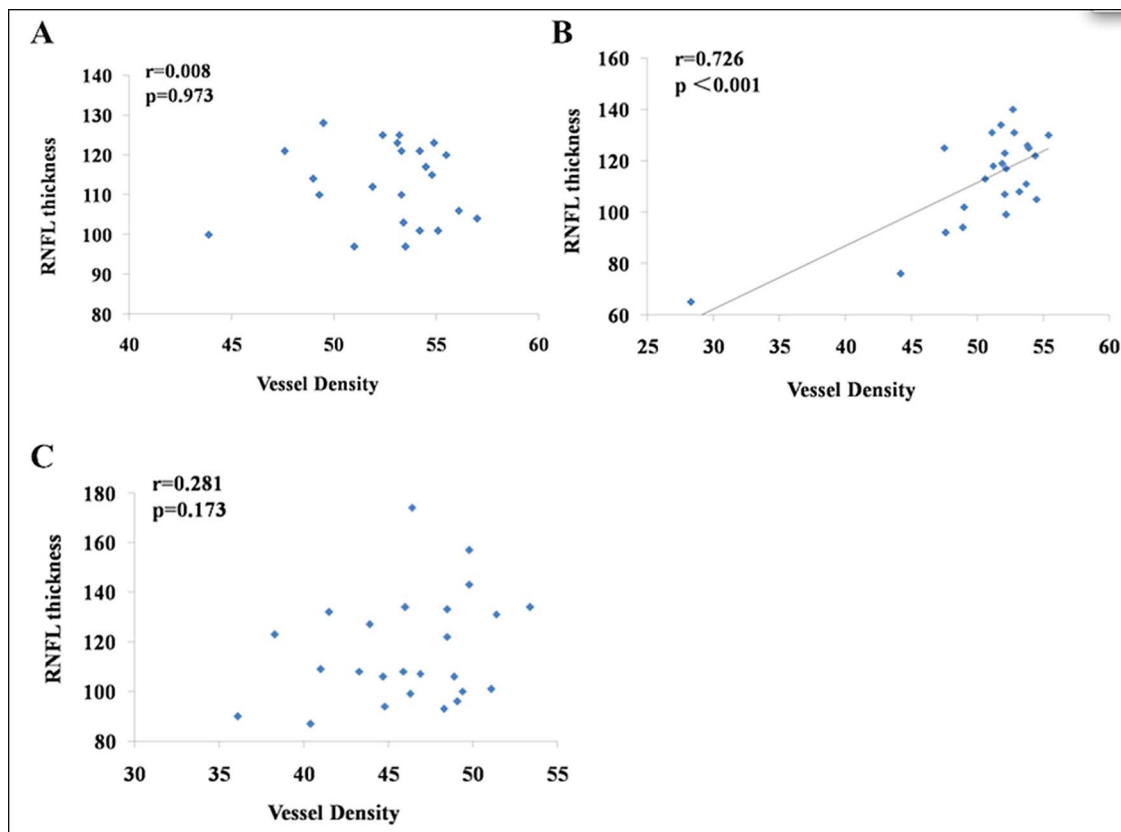

Figure 5. Scatterplots illustrating the linear associations between vessel density and RNFL thickness of the entire peripapillary region in the no DR group (A), mild DR group (B), and the severe DR group (C). A value of $P < 0.05$ was considered statistically significant. Correlation coefficient from the Spearman rank correlation analysis.

have demonstrated an association between RPC changes and RNFL loss in glaucoma.[20–22] Given the involvement of RPCs in the structural and functional changes in conditions, such as glaucoma, cotton wool spots, Bjerrum scotoma, and ischemic optic neuropathy,[10–12] studies involving patients with diabetes to investigate how RPCs and the RNFL may be influenced in such circumstances may offer further insights.

Quantitative evaluation of RPCs was limited by the difficulties of visualizing the vessels on conventional fluorescein or indocyanine green angiography as a result of choroidal circulation.[23,24] OCTA provides a noninvasive and fast approach to peripapillary region perfusion analysis[13,24] as it is a transition from structural to functional imaging. Many studies have shown that OCTA allows clear capillary definition around the optic papilla[9,20–22] and is highly repeatable in follow-up among operators.[13]

Currently, there is very limited quantitative data available regarding peripapillary region microcirculation in diabetic patients. We found a significant decrease in vessel density of RPC in the peripapillary region in patients with DR, which was similar to the results of a recent publication by Vujosevic et al.[25]; however, Vujosevic et al.[25] did not report the data of eyes with moderate or severe NPDR or PDR. In our study, we found a significant correlation between DR severity and vessel density in the peripapillary region, which differed from the findings of Vujosevic et al.[25] and Cao et al.[26] who observed a decrease in vessel density of RPC in the peripapillary region in patients with DM but without DR. These results may have differed, due to the difference in demographics or the use of different OCTA technologies.

Previous studies have demonstrated RNFL thinning in patients in the early stages of diabetes[27,28] as we observed in the RNFL of diabetic patients without DR in our study. The lack of significant difference in RNFL thickness in the peripapillary region between the no DR, mild DR, and severe DR groups observed here could possibly be attributed to the structural changes in the retinal tissue in the peripapillary region caused by intracellular and extracellular edema, hemorrhage, exudation, or glial fibrillary degeneration around the optic papilla.[29,30] Additionally, most previous studies used OCT to analyze RNFL thickness changes at early stages of DR.[27,28] Notably, we did not find a correlation between RNFL thickness changes in the peripapillary region and disease severity in eyes with DR via OCTA. It might be a clinical challenge to evaluate RNFL impairment by assessing the thickness in patients with severe DR, further emphasized by our correlation analysis findings between the vessel density and the RNFL thickness in the context of increasing DR severity. A statistically significant decrease in the RNFL thickness was observed in the no DR group when compared with the control group, which was similar to previously published results.[27,28] However, no significant difference in the vessel density was observed between the control group and the no DR group.

Interestingly, there was a significant positive correlation between vessel density and RNFL thickness of the peripapillary region in the mild DR group. It demonstrated that the decreased RPC perfusion caused the impairment of nutrition supply to the RNFL to be impaired, which might have a profound influence on the activity and metabolism of the nonmyelinated axons located in the RNFL. The decrease of vessel density in the peripapillary region can be attributed to microvascular impairment. During diabetes progression, structural alterations of capillaries occurs, including vascular basement membrane thickening, endothelium dysfunction, and pericyte apoptosis, and these changes would cause a reduction in blood flow and capillary occlusion.[31,32] These structural alterations of capillaries also occur in small blood vessels in the peripapillary region.[33] When the RPC perfusion decreased further, structural changes in the RNFL, such as intracellular and extracellular edema and hemorrhage, caused RNFL thickness to increase when assessed by OCTA. This may explain why no statistically significant associations were found between vessel density and RNFL thickness of the peripapillary region in the severe DR group.

A limitation of this study is that it is difficult to deduce the causal relationship between microcirculation changes in the peripapillary region and neurodegenerative changes from this cross-sectional study. In future studies, an improvement would be to make longitudinal observations to analyze the relationship between optic papilla blood circulation changes and RNFL layer thickness changes in DR patients throughout disease progression. Another limitation includes the absence of vessel skeletonization, which can remove the influence of vessel size on retinal perfusion measurements.[25] Moreover, due to severe macular edema in some patients with severe DR, eyes with diabetic macular edema (where there is swelling of the RNFL) are prone to segmentation errors. This may have led to inaccurate vessel density measure-

ments, despite accounting for this by manually correcting the entire scan volume.

In conclusion, vessel density of RPCs for the peripapillary region was closely related to DR severity and decreased with DR progression. Nevertheless, there were no obvious correlations observed between changes in RNFL thickness and DR severity, suggesting that careful monitoring of RPC vessel density in the peripapillary region might reveal neurodegeneration during clinical stages of DR. These findings may provide valuable insights, thus heightening our understanding, and offer a new direction for further investigation into microvasculature and neurodegeneration in DR.

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