

# Early Neurovascular Changes in the Retina in Preclinical Diabetic Retinopathy and Relation to Blood Glucose

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## Research article

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# Abstract

**Aim:** To investigate the changes of retinal optic disc nerve fiber layer thickness and macular blood flow density in preclinical stage of diabetic retinopathy and their relationship with blood glucose.

**Methods:** In this cross-sectional study, 97 diabetic patients (total 188 eyes, 144 eyes in no diabetic retinopathy group, 44 eyes in mild diabetic non-proliferative retinopathy group) and 35 healthy people (70 eyes) were enrolled. All the subjects were divided into different group by HbA1c, and underwent ocular examination by optical coherence tomography angiography. We compare optical coherence tomography angiography parameter and retinal nerve fiber layer thickness among different glucose group.

**Results:** The parafoveal vessel density and the temporal retinal nerve fiber layer thickness were lower ( $P < 0.05$ ) in the diabetic group than in the normal group. The diabetic group showed a higher acircularity index as compared with the normal group. From the normal group to no diabetic retinopathy group and then to mild non-proliferative retinopathy group, vessel density decreased and acircularity index increased ( $P < 0.001$ ). Foveal vascular density and parafoveal vessel density decreased with the increase of HbA1c. There was a negative correlation between parafoveal vessel density of the deep retinal vascular layer and FBG ( $P < 0.01$ ). The temporal retinal nerve fiber layer thickness decreased among different HbA1c levels groups and was positively correlated with the parafoveal vessel density in superficial retinal vascular layer ( $P < 0.05$ ).

**Conclusions:** This study shows retinal microvasculopathy and neuropathy has been present during no retinopathy. The vessel density of the deep retinal vascular layer was negatively correlated with fasting blood glucose, and the temporal RNFL thickness was positively correlated with the vessel density of superficial retinal vascular layer. These indicators are helpful for endocrinologists and ophthalmologists to detect early diabetic retinal pathological lesions.

## 1. Introduction

Diabetic retinopathy (DR) is a diabetes-specific microangiopathy, which is one of the main causes of blindness in the world. It affects more than one-third of diabetic patients and gradually becomes a worldwide public health challenges [1-3]. Recent studies have found that DR has both microvasculopathy and neuropathy. The neurodegenerative changes of DR include apoptosis of retinal neurons and the thinning of retinal nerve fiber layer thickness (RNFL) [4, 5]. RNFL is composed of ganglion cell axons in the retina, which is located at the innermost end of the retina. The change of RNFL thickness can reflect the injury of optic nerve. The microangiopathy of DR can be manifested by the decrease of capillary density in the macular area. The macular fovea is the most acutely visual area, which is surrounded by a capillary arch. The central avascular area is called foveal avascular zone (FAZ), which is very important for maintaining fine vision. The changes in the morphological area of FAZ and the vessel density of the surrounding capillary network can reflect the degree of retinal ischemic disease. Optical coherence tomography angiography (OCTA), the latest ophthalmic instrument, can simultaneously display the morphology and density of macular vessels and measure the RNFL thickness. The purpose of this study was to investigate the changes of RNFL thickness in retinal optic disc and macular blood flow density in preclinical stage of diabetic retinopathy and their relationship with blood glucose.

## **2. Materials And Methods**

### **2.1 Participants**

This cross-sectional study was performed at the endocrinology department of Beijing Hospital and was approved by the Institutional Ethics Committees of Beijing Hospital. In the study, participants were consecutively recruited from February 2018 to December 2019. The subjects signed the informed consent form, and any accompanying image was obtained from all participants. The diagnosis of type 2 diabetes mellitus was based on the 1999 WHO standard. The inclusion criteria were as follows: (1) the best corrected visual acuity was not worse than Snellen 20/200; (2) the intraocular pressure was within the normal range; (3) there was no history of external injury and internal and external eye surgery; and (4) there were no other eye lesions except mild cataract. Exclusion criteria: (1) age-related macular degeneration, (2) congenital maculopathy, (3)macular epiretinal membrane, (4) keratopathy, lens opacity, vitreous liquefaction or hemorrhage, (5) patients with other non-diabetic chorioretinopathy (eg: hypertensive retinopathy), (6)Refractive error >3.00 D, (7) severe non-proliferative diabetic retinopathy(NPDR )and proliferative diabetic retinopathy(PDR), (8) retinal choroiditis and uveitis, (9) glaucoma, (10)retinal arteriovenous occlusion, (11) photocoagulation or eye surgery. Initially a total of 117 patients with type 2 diabetes were enrolled, 20 patients with incomplete data were excluded. Finally data of 97 patients were included in the statistical analysis. Among them, 62 were male and 35 were female, with an average age of (54 ±10) years, fasting blood glucose (FBG) 8.0 ±2.6 mmol/L, HbA1c 9.1 ±1.9%. The normal control group was 35 healthy staffs and volunteers without diabetes and ophthalmological diseases. There were 10 males and 25 females, with an average age of (53 ±11) years, FBG 5.2 ±0.4 mmol /L, HbA1c 5.6 ±0.3%. According to different HbA1c levels, the patients were divided into four groups:1. $\leq$ 6.1%(mean 5.6±0.3%);2. 6.1~8.0%(mean 7.3±0.5%); 3.8.0~10%(mean 9.1±0.5%);4.>10%(mean 11.9±1.3%), respectively.

### **2.2 Ocular Examination**

Fundus routine mydriasis examinations including simple funduscopy and fundus photographs were performed by fixed ophthalmologists, according to early DR treatment study (Early Treatment Diabetic Retinopathy Study, ETDRS) international DR staging standard [6] to assess fundus lesions, excluding proliferative DR, moderate and severe non-proliferative DR (NPDR) and macular edema. Each patient underwent intraocular pressure measurement .They were divided into no-DR group and mild NPDR group.

### **2.3 Optical Coherence Tomography Angiography Imaging**

All OCTA examinations were performed by the same skilled operator. The examiners take the sitting position and adjust their eyes to the appropriate position without dilating the pupil. OCTA imaging of the macular area was performed by using an Angio Vue OCTA device ( Optovue, Inc, RTVue XR), which has an A-scan rate 70,000 scans per second, using a wavelength of 840 nm and a bandwidth of 45nm, Each OCTA volume contains 304×304 A-scans with two consecutive B-scans captured at each fixed position .The scanning area was captured in 3- ×3-mm sections centered on the fovea ,and two orthogonal OCTA scans were acquired to minimize motion artifacts.

The newly developed, built-in AngioAnalytics software (version 2017.1.0.155; Optovue, Inc.) was used to quantitatively evaluate vessel density (VD) of superficial and deep capillaries of the retina-FAZ area size and RNFL thickness. An image quality index (QI) ranged from 0 to 10 was given by the software for each scan. The capillary plexus in macular region was automatically stratified into two layers: the superficial retinal vascular layer extending from the inner limiting membrane to the inner plexiform layer with an offset of 10 mm, the deep retinal vascular layer reaching from the inner plexiform layer with an offset of 10 mm to the outer plexiform layer with an offset of 10 mm. The whole VD means the vessel density of the whole image. The parafoveal region is defined as a 3.0 mm-wide round annulus around the fovea 1.0 mm circle. The foveal vascular density (FD-300) was determined as vessel density within a ring of a width of 300  $\mu\text{m}$ -wide regions around the FAZ. Measurements of the FAZ were generated based on the Retina slab with automated detection of the FAZ boundary by the AngioVue software. The acircularity index (AI) was calculated as the ratio of the FAZ perimeter divided by the perimeter of a circle with equal area. Taking the optic disc as the center, RNFL thickness measurement makes a circular scan with a diameter of 3.46 mm, including the superior, the inferior, and the nasal and temporal side of the optic disc. All analyses were performed by the same inspector.

There were 188 eyes in the diabetic group, including 144 eyes in the no-DR group and 44 eyes in the mild NPDR group, and the other 6 eyes were not further grouped because the QI was less than 6. There were 70 eyes in the normal control group.

## 2.4 Statistical Analysis

Statistical analyses were performed using SPSS software for Windows, version 16.0. All data are shown as the mean  $\pm$  standard deviation ( $x \pm s$ ). Differences in the data were assessed using the independent t-test or analysis of variance (ANOVA) and pairwise comparison was made by one-way ANOVA test with post hoc pairwise comparison. Spearman rank correlation analysis was used to analyze the correlation between VD and blood glucose and RNFL.  $P < 0.05$  was considered statistically significant.

# 3. Results

## 3.1 Patients Characteristics

This study included 188 eyes of 97 type 2 diabetes subjects. Six eyes were excluded on account of QI less than 6. There were 144 eyes (72 patients) in no-DR group, 44 eyes (25 patients) in mild NPDR group. Seventy eyes of 35 age matched healthy controls were also included. The mean age of the control group was  $53 \pm 11$  years; the no-DR group  $53 \pm 10$  years; and the mild NPDR group,  $57 \pm 9$  years. The mean duration of diabetes was  $9.2 \pm 6.0$  years for the no-DR group and  $14.4 \pm 9.1$  years for the mild NPDR group ( $p < 0.05$ ). There was significant difference in the HbA1C between the 2 groups ( $9.1 \pm 1.9\%$  versus  $5.6 \pm 0.3\%$ , in diabetic and control group  $p < 0.001$ ). Systolic blood pressure(SBP) in diabetic group was higher than that in control group  $128 \pm 19\text{mmHg}$  versus  $116 \pm 8\text{mmHg}$ ,  $p < 0.001$ ). There was no difference in creatinine and total cholesterol between diabetic group and control group. (Table 1)

**Table 1:** Characteristics of the Study Participants

	DM				Control group			
	no-DR (N=72)	mild- NPDR (N=25)	t	p*	Total (N=97)	N=35	T	P**
Age(years)	53±10	57±9	1.725	0.088	54±10	53±11	0.586	0.559
DM duration (years)	9.2±6.0	14.4±9.1	2.694	<b>0.011</b>	10.5±7.3	NA	14.169	<b>&lt;0.001</b>
SBP(mmHg)	127±12	136±19	2.386	<b>0.023</b>	128±19	116±8	5.017	<b>&lt;0.001</b>
HbA1c(%)	8.9±1.8	9.5±2.2	1.186	0.239	9.1±1.9	5.6±0.3	9.102	<b>&lt;0.001</b>
Fasting blood Glucose(mmol/l)	8.1±2.7	7.9±2.6	-0.391	0.696	8.0±2.6	5.2±0.4	9.728	<b>&lt;0.001</b>
Cr(umol/l)	60.2±24.8	70.7±32.0	1.658	0.101	63±27	55±11	1.463	0.146
TC(mmol/l)	4.1±1.0	4.1±1.1	0.001	1.0	4.1±1.1	4.4±0.9	-1.584	0.116

SBP:Systolic blood pressure; Cr: creatinine; TC: total cholesterol.

\* Comparison between the no-DR and DR groups with independent samples t-test.

\*\* Comparison between the DM (total) and control groups with independent samples t-test

### 3.2 OCTA parameter and RNFL thickness in diabetic group were compared with those in normal control group.

The diabetic group showed a significantly ( $P < 0.001$ ) lower VD in the superficial and deep retinal vascular layer in the parafoveal and the whole region as compared with the normal group. Additionally, the FD-300 was significantly ( $P < 0.001$ ) lower in the diabetic group than that in the normal group. The AI value in the diabetic group was significantly higher than that in the normal control group. The area of FAZ in the diabetic group was slightly larger than that in the normal control group, but there was no significant difference between the two groups ( $P = 0.583$ ). From the normal control group to the no-DR group and then to the mild NPDR group, the VD of the superficial and deep retinal vascular layer and FD-300 decreased gradually, and there was significant difference among the three groups. The comparison of one-way ANOVA test with post hoc pairwise comparison showed that there were significant differences each groups ( $P < 0.001$ ). There was no significant difference in the size of FAZ ( $P = 0.659$ ) among three groups. AI increased gradually, and there was significant difference among the three groups ( $1.14 \pm 0.05$  vs.  $1.16 \pm 0.06$  vs  $1.20 \pm 0.08$ ,  $F=13.101$ ,  $P<0.001$ ). (Table2,Figure1).

The thickness of RNFL in the superior and temporal side of optic disc were significantly lower in the diabetic group than that in the normal group( $p<0.05$ ). Interestingly, the temporal RNFL thickness of optic disc in the

normal group and mild NPDR group was significantly larger than that in the no-DR group, but there was no significant difference in superior, inferior and nasal RNFL thickness. (Table 3, Figure 1).

Because of the significant difference of SBP among groups, we used SBP as covariate, grouping as fixed factor, VD and RNFL as dependent variables. The results showed that SBP had no significant effect on VD and RNFL. (Table 4)

**Table 2:** Comparisons of OCTA parameter in retina between normal group and diabetes with or without retinopathy group.

Number	DM			Control group			
	no-DR (N=144)	mild- NPDR (N=44)	P*	Total (N=188)	N=70	P**	p***
Superficial Whole VD (%)	44.4±4.1	40.4±5.2	<0.001	43.4±4.7	46.5±2.6	<0.001	<0.001
Superficial parafoveal VD(%)	47.3±4.4	42.8±5.5	<0.001	46.3±5.1	49.4±3.1	<0.001	<0.001
Deep whole VD(%)	49.1±3.7	45.3±4.5	<0.001	48.2±4.2	51.2±7.1	<0.001	<0.001
Deep parafoveal VD(%)	51.7±3.9	47.6±5.0	<0.001	50.7±4.5	53.8±7.4	<0.001	<0.001
FD-300(%)	47.7±4.6	43.5±5.4	<0.001	46.8±5.1	51.2±3.5	<0.001	<0.001
FAZ Area(um <sup>2</sup> )	0.35±0.17	0.38±0.13	0.466	0.36±0.16	0.37±0.28	0.583	0.659
AI	1.16±0.06	1.20±0.08	<0.001	1.17±0.06	1.14±0.05	<0.001	<0.001

VD: vessel density, FD-300: the foveal density, AI: the acircularity index

\*Comparison between the no-DR and mild-NPDR groups by One-way ANOVA test with post hoc pairwise comparison .

\*\*Comparison between the DM (total) and control groups by independent samples t-test

\*\*\*Comparison among the no-DR, mild-NPDR and control groups by one-way ANOVA test

**Table 3:** Comparisons of RNFL thickness in disc between normal group and diabetes with or without retinopathy group.

DM				Control group			
	no-DR (N=144)	mild-NPDR (N=44)	p*	Total (N=188)	N=70	p**	p***
Temporal (um)	72.5±10.4	78.6±12.9	<b>0.001</b>	73.9±11.3	78.1±10.6	<b>0.008</b>	<b>&lt;0.001</b>
Nasal(um)	76.2±11.8	76.8±12.3	0.802	76.4±11.9	74.7±16.9	0.459	0.665
Superior (um)	122.8±16.7	122.5±22.1	0.918	122.7±18.1	128.4±15.3	<b>0.020</b>	0.068
Inferior (um)	129.5±15.8	129.9±19.9	0.897	129.6±16.8	132.9±19.1	0.171	0.389

Temporal: the RNFL thickness in temporal field of disc; Nasal: the RNFL thickness in nasal field of disc; Superior: the RNFL thickness in superior field of disc; Inferior: the RNFL thickness in inferior field of disc

\*Comparison between the no-DR and mild-NPDR groups by One-way ANOVA test with post hoc pairwise comparison.

\*\*Comparison between the DM (total) and control groups by independent samples t-test

\*\*\*Comparison among the no-DR, mild-NPDR and control groups by one-way ANOVA test

**Table 4:** Covariance result of VD and RNFL

Dependent variable	Fixed factor	Covariate	p*	p**
Superficial Whole VD%	Group (DM vs Control)	SBP	<b>&lt;0.001</b>	0.533
Superficial parafoveal VD(%)			<b>&lt;0.001</b>	0.491
Deep whole VD(%)			<b>&lt;0.001</b>	0.525
Deep parafoveal VD(%)			<b>&lt;0.001</b>	0.618
FD-300(%)			<b>&lt;0.001</b>	0.861
AI			<b>&lt;0.01</b>	0.673
Temporal (um)			<b>&lt;0.05</b>	0.997
Superior (um)			<b>&lt;0.05</b>	0.634

\*The independent effect of fixed factor on dependent variables.

\*\*The effect of covariate on dependent variables.

### 3.3 Analysis of OCTA parameter and RNFL thickness in different blood glucose level group

After dividing into four groups according to HbA1c levels, we observed that with the increase of HbA1c, the decrease of FD-300 and VD gradually from the first group to the third group, but VD and FD-300 in the fourth group was slightly higher than those in the third group. Multiple comparisons revealed statistically

significant differences in those OCTA parameters between the first group and the other three groups ( $P<0.01$ ), but no statistically significant differences between the other three groups ( $P>0.05$ ). The AI in the first group was significantly smaller than the other three groups( $P<0.01$ ). There was no significant difference in FAZ size between those groups( $P>0.05$ ). The temporal RNFL thickness of optic disc decreased gradually among groups with different HbA1c levels, but abnormally increased in group 3 (Table 5).

The Spearman correlation analysis showed there was a significant negative correlation between parafoveal whole vessel density of the deep retinal layer and FBG ( $r = -0.170\text{--}0.163$ ,  $P=0.006$ ,  $P=0.009$  respectively). RNFL thickness in temporal optic disc was positively correlated with the parafoveal VD in superficial layer( $r=0.131\text{--}P<0.05$ ).

**Table 5:** Comparisons of OCTA parameter of retina and the RNFL thickness of disc in 4 groups of the different HbA1c level.

	Group1 (≤6.1%)	Group2 (6.1%~8.0%)	Group3 (8.0%~10%)	Group4 (>10%)	p***
Number	82	67	65	44	
Superficial Whole VD(%)	46.2±2.9*	43.9±4.4	42.9±5.2	43.2±4.5	<b>&lt;0.001</b>
Superficial parafoveal VD(%)	49.1±3.3*	46.9±4.8	45.4±5.5	46.1±4.8	<b>&lt;0.001</b>
Deep whole VD(%)	51.2±6.5*	48.1±3.2	47.7±4.8	48.6±4.9	<b>&lt;0.001</b>
Deep parafoveal VD(%)	53.7±6.9*	50.7±3.2	50.4±5.8	51.1±5.3	<b>&lt;0.001</b>
FD-300(%)	50.8±3.8*	46.9±5.0	45.9±5.1	47.4±5.2	<b>&lt;0.001</b>
FAZ Area(um <sup>2</sup> )	0.37±0.26	0.38±0.20	0.32±0.12	0.36±0.15	0.388
AI	1.14±0.05*	1.17±0.07	1.17±0.08	1.17±0.05	<b>0.002</b>
Temporal (um)	76.6±10.7**	73.7±9.0	77.6±13.5**	71.5±11.2	<b>0.036</b>
Nasal(um)	74.3±15.7	77.5±12.1	75.8±13.2	77.2±10.8	0.471
Superior (um)	127.1±14.8	123.9±19.7	123.8±17.7	119.7±18.0	0.165
Inferior (um)	131.7±18.2	129.5±18.5	130.1±16.9	129.9±15.8	0.921

\*Comparison between the group1 and other groups by One-way ANOVA test with post hoc pairwise comparison with  $p<0.01$

\*Comparison between the group4 and other groups by One-way ANOVA test with post hoc pairwise comparison with  $p<0.05$

\*\*\*Comparison among 4 groups by one-way ANOVA test

## Discussion

In this study, we analyzed the changes of macular blood flow density, FAZ area and optic disc RNFL thickness by OCTA in normal control group, no-DR group and mild NPDR group, and whether they were correlated with blood glucose level. Diabetic retinopathy is a specific diabetic microvascular complication. Hyperglycemia leads to retinal microvascular disorder, non-perfusion in capillary plexus and macular ischemia. In the past, we found microaneurysms by fundus fluorescein angiography to diagnose early diabetic retinopathy, but many recent studies have shown that the retinal capillaries have changed before the clinical diagnosis of diabetic retinopathy, including the decrease of parafoveal VD and irregular FAZ[7-8,10]. Our research results are consistent with those. Comparison with the control group, the VD of superficial and deep retinal layer and FD-300 were significantly lower in the no-DR group. The result suggested even if diabetic retinopathy has not been diagnosed clinically, the blood flow of the retinal capillary network, especially the blood density around the fovea, was impaired, which represented the ischemic change of low perfusion in preclinical diabetic retinopathy stage. Previous studies have also suggested that parafoveal VD has a good correlation with diabetic microvascular disease[11], which may be used as a marker of pre-diabetic retinopathy[10].

As for the changes of FAZ area in diabetic patients, the results are not consistent. Some studies have shown that there is no significant change in FAZ area in diabetic patients without retinopathy [7-8,10], while other studies have found that FAZ area in diabetic patients without retinopathy is significantly higher than that in normal people [12-15]. Previous studies have shown that there are individual differences in FAZ area [16,17], and the AUC area of FAZ is small, so it is difficult to evaluate whether FAZ is pathologically enlarged by area measurement [18]. Therefore, FAZ area may not be a sensitive marker for the diagnosis of early diabetic retinopathy. The irregular shape of FAZ caused by the decrease of capillary network perfusion and the macular ischemia may better reflect the ischemia in the fovea. Fang used the FAZ circularity index to evaluate FAZ shape, the result showed FAZ circularity index decreased in DR[19]. AI is a new method to evaluate the degree of capillary injury in FAZ area, which reflects the morphological change of FAZ and quantitatively analyzes the degree of capillary circulation around fovea by the tortuously change of the FAZ perimeter. Compared with other FAZ indexes, AI can better reflect the irregularity of FAZ morphology. The results of our study is first to show that the AI increased gradually from the normal control group to the no-DR group and then to the mild NPDR group, indicating that the FAZ morphology of the no-DR group had changed irregularly, and the FAZ morphology of the mild NPDR group was more irregular, which indicated the aggravation of ischemia.

Blood glucose level affects retinal blood VD. The study has shown that low VD around optic disc is related to high FBG[8]. Lavia found a rapid decrease in macular VD was observed over 12 months and were associated with a rapid improvement in blood glucose level[9]. Among the groups with different HbA1c levels, we found that there were significant differences in VD between group 1 (HbA1c< 6.1%) and other groups, but there was no significant difference among other groups. It was speculated that once the blood glucose level increased, the retinal microcirculation showed significant blood flow changes, and then with the increase of blood glucose level, the VD further decreased, but the change was not significant. The results suggest that the initial stage of elevated blood glucose may be the most effective time for intervention. But our results need more sample size to confirm. OCTA parameter alterations in the deep vascular layer are more serious than in the superficial vascular layer in NPDR [20-23], especially in patients with DME [24-26]. Our results showed

that deep retinal VD in macular area was negatively correlated with FBG level, while VD in the superficial layer of the retina is not correlated, suggesting that deep retinal VD was more closely related to the change of blood glucose.

The neurons, ganglion cell layer and nerve fiber layer of the inner retina are closely connected with the extensive capillary network and form functional neurovascular units together with glial cells, pericytes and capillary endothelial cells. The nerve fiber layer is composed of axons of optic ganglion cells, which are mostly unmyelinated axons, which require more energy and are more vulnerable to ischemic damage [27]. The blood supply to these nerve tissues mainly comes from the superficial capillary network of the retina. In the preclinical DR and early stages of diabetic retinopathy, microvascular changes are also accompanied by retinal neurodegenerative and neuroelectrophysiological changes,[4,5,10]. Thinning of RNFL thickness and GC-IPL thickness suggests neurodegenerative changes and are related to diabetic retinopathy [28, 29]. Although one study showed there was no significant changes of average or sectoral RNFL thicknesses in no-DR[30] , some other studies found RNFL loss may occur in no-DR[31,32].Our study showed that the temporal RNFL thickness in diabetic patients was significantly thinner than that in the normal group. Further analysis revealed that the temporal RNFL thickness in the no-DR group was significantly smaller than that in the normal group. The decrease of RNFL thickness indicates that the retinal nerve tissue has degenerative changes in preclinical DR stage. We also found the temporal RNFL thickness of optic disc was only positively correlated with parafoveal VD in the superficial retina vascular layer. RNFL is located in the superficial capillary network of retina, and the macular fovea is located in the temporal side of optic disc, so the change of parafoveal VD in the superficial retina of macular area may affect the temporal RNFL thickness of the optic disc. This suggests that microangiopathy and neuropathy work together on retinopathy and are related to each other.

Poor blood glucose control can lead to the decrease of RNFL thickness. Study has shown that poorer glucose tolerance was significantly associated with the reduction of circumpapillary RNFL, but did not show a significant correlation with HbA1c.[33]. In our study, there was a significant difference in the temporal RNFL thickness among different HbA1c levels groups, which decreased gradually, but abnormally increased in group 3. There was no significant correlation between RNFL thickness and HbA1c. The local leakage was aggravated with the development of hyperglycemia. If severe microvascular lesion such as macular edema occurs, it is more likely to show damage to the deep capillary network of the retina [24].and the intracellular and extracellular edema would cause the abnormal increase of RNFL thickness . That result in no significant correlation between HbA1c level and RNFL thickness .

There are also some limitations in this study. Firstly, this study is a small cross-sectional clinical study, which is not comprehensive enough and needs to be further confirmed by a larger sample size. Secondly, although the changes of retinal VD and RNFL thickness were observed at the same time, and the relationship between VD and blood glucose levels was observed, it is still not clear that the correlation between RNFL thickness and blood glucose changes. That may be due to our sample size is not large enough.

In summary, our studies have shown that there was a decrease in macular vessel density and a thinning of the temporal RNFL of the optic disc during preclinical DR stage. From the normal group to the no-DR group and then to the mild NPDR group, the macular superficial and deep VD decreased and AI increased gradually,

respectively. The macular deep VD was negatively correlated with FBG, while the temporal RNFL thickness of the optic disc was positively correlated with the superficial retinal VD of the macular . It is suggested that we should pay attention to the early changes of retinal microvascular and neurodegeneration in T2DM without DR. Indicators such as macular VD, AI and the temporal RNFL thickness of optic disc can help us to detect early pathological changes. The changes of VD at different blood glucose levels may suggest the importance of early intervention and treatment.

## Declarations

### DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Ethics Committees of Beijing Hospital.Written informed consent of the study was received from all subjects.

### FUNDING SOURCE

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### CONSENT FOR PUBLICATION

Not applicable.

### FINANCIAL DISCLOSURE

All the authors have no financial relationship relevant to this article to disclose.

### CONFLICT OF INTEREST

All the authors have no conflicts of interest to disclose.

### AUTHORS' CONTRIBUTIONS

Concept and design: HL, XBY and LXG;

Data acquisition: HL, BDZ, SD and ZQM;

Data analysis / interpretation: HL, XBY, BDZ and LXG;

Draft the manuscript: HL;

Critical revision of the manuscript: CYS, XBY, BDZ, SD, ZQM and LXG;

Supervision: XBY.

All authors read and approved the final manuscript.

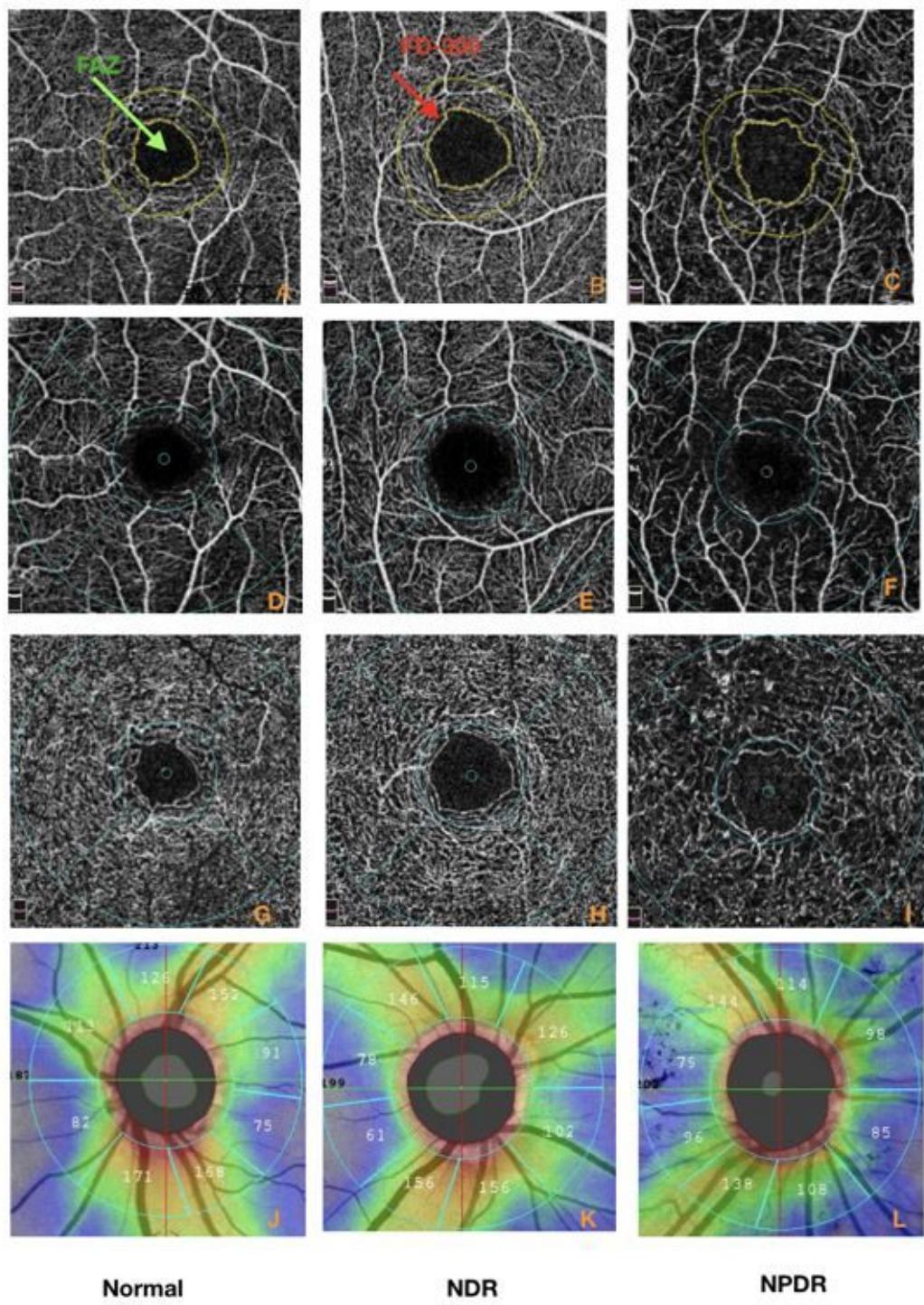
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## Figures



**Figure 1**

Representative sample of vessel densities in the macula region and RNFL thickness of optic disc for each group (control, NDR, mild NPDR). Green arrow indicate FAZ, red arrow indicate FD-300. Top row: the size and shape of FAZ, FD-300; Second row: VD of the superficial retinal vascular layer in macular area; Third row: VD of the deep retinal vascular layer in macular area; Bottom row: RNFL thickness in optic disc.