

Macular and peripapillary vessel density measurement in the evaluation of Non-proliferative Diabetic Retinopathy

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Research article

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Abstract

Purpose: To compare vessel density in macular and peripapillary area between control subjects and patients with non-proliferative diabetic retinopathy (NPDR) using optical coherence tomography angiography (OCTA) and to evaluate the association between RNFL thickness and different stage of diabetic retinopathy.

Methods: A total of 170 eyes (normal control, 43; mild NPDR, 43; moderate NPDR, 42; severe NPDR, 42) underwent OCTA imaging. Optical coherence tomography angiographic parameters were vessel densities of superficial capillary plexus (SCP), deep capillary plexus (DCP) in macular area and peripapillary area.

Results: Vessel density of SCP and DCP in macular area, peripapillary area as well as RNFL thickness were $53.13\% \pm 3.03\%$, $52.07\% \pm 2.28\%$, $53.78\% \pm 3.66\%$ and $128.86\mu\text{m} \pm 11.32\mu\text{m}$, respectively, in control subjects; $50.24\% \pm 3.81\%$, $47.40\% \pm 4.50\%$, $46.50\% \pm 6.03\%$ and $124\mu\text{m} \pm 13.97\mu\text{m}$, respectively, in mild NPDR; $46.80\% \pm 5.23\%$; $44.39\% \pm 3.99\%$; $44.64\% \pm 4.23\%$; $121.02\mu\text{m} \pm 20.86\mu\text{m}$, respectively, in moderate NPDR; $42.82\% \pm 5.46\%$, $42.34\% \pm 5.14\%$, $43.16\% \pm 4.47\%$, $118.60\mu\text{m} \pm 21.91\mu\text{m}$ in severe NPDR. The reduction of vessel density of SCP and DCP in macular area, peripapillary area as well as RNFL thickness were correlated with increasing severity of DR. Vessel density of SCP and DCP in macular area, peripapillary area and foveal density 300 (FD 300) in normal control were significantly higher than that of mild, moderate and severe NPDR groups. (all $P < 0.001$). Vessel density of DCP shows better ability to identify the severity of DR (0.913; 95% CI=0.867-0.958; cut off value:0.75) than FD 300, vessel density of SCP in macular area and peripapillary area.

Conclusion: Macular and peripapillary vessel density as well as RNFL thickness decreased as DR progresses. Vessel density in DCP could be an objective and sensitive indicator for monitoring progression of DR. OCTA might be clinically useful to evaluate microvascular and microstructural alterations in macula and optic nerve head (ONH), thus providing a new method to study the course of DR.

Introduction

Diabetic retinopathy is known as a microvascular complication of diabetes mellitus and the leading cause of blindness in economically active people worldwide. [1, 2] Fluorescein angiography (FA) is the gold standard for diagnosing and staging DR yet it is invasive and might cause several side effects.[3, 4] Optical coherence tomography angiography (OCTA) is a current alternative non-invasive angiographic technique which can demonstrate a 3-dimensional vascular mapping at the microcirculation level.[5] With the utilization of OCTA, visualization of morphology of retinal and choroidal vessels and identification of ischemic area on a layer-by-layer basis became possible.[6] The recent appliance of OCTA can also readily quantified optic nerve head (ONH) microcirculation as well as RNFL thickness.[7–9]

Many researches have used OCTA to indicate that vessel density in capillary plexus and choriocapillaries reduced as DR progressed.[10–12] However, seldom did studies combine the analysis of macular area

and optic disc in one research. It is noteworthy that, instead of focusing changes on macular perfusion solely, analyzing peripapillary perfusion might have important meaning on certain patients, such as DR patients with glaucoma, or DR patients with other fundus diseases that might affect macular perfusion. Simply relying on alteration of vessel density in macula might risk wrong judgement to patients' condition. Hence, to measure vessel densities of both optic disc and macular area might actually provide a more comprehensive assessment on DR patients even though these two microcirculations origin from different sources. More importantly, more and more researches document DR as a neurovascular disease as evidence showed prominent decreased RNFL thickness in early stage of DR as well[13, 14]. The term 'neurovascular unit' has gaining attentions[15, 16] and OCTA might indeed shed some light on this matter because it not only could measure vessel density but also RNFL thickness.

Hence, in this study, we aim to investigate macular and peripapillary vessel densities, and RNFL thickness between normal subjects and diabetic patients with different stages of non-proliferative diabetic retinopathy (NPDR) using OCTA and to analyze the relation among these indicators, so that we could further our understanding towards the development of DR in terms of microcirculation and microstructure.

Methods

Subjects

We recruited patients with type 2 diabetes mellitus (T2DM) and healthy control subjects who presented between January 2017 to January 2018 from Guangdong General Hospital. Written informed consent was obtained from all participants. A single eye was randomly selected if both were eligible. Exclusion criteria for all participants were as follows: any other ocular disease that may affect ocular circulation (e.g. glaucoma, age-related macular degeneration, retinal vascular occlusion, refractive error >3 diopters[D]), intraocular surgery, hypertension exceeding 150/100mmHg, intraocular pressure (IOP) >21mmHg. Individuals with a family history of glaucoma were also excluded in the present study. All participants were tested for best IOP, corrected visual acuity (BCVA), and refractive error (autorefractometry). Slit-lamp and fundus examinations using direct and/or indirect ophthalmoscope were performed. ETDRS 35 degree 7-standard fields color retinal photographs (Topcon TRC; Topcon, Tokyo, Japan) was required for each participant. DR was graded according to the International Diabetic Retinopathy Severity Scale.

Fundus photographs evaluation and stage classification were done by two experienced graders (DC and ZNH).

Optical coherence tomography angiogram acquisition and analysis

Optical coherence tomography angiography was performed using AngioVue Optical coherence tomography angiography system (RTVue-XR Avanti; Optovue, Fremont, CA, USA, version 2017.1.0.151). Each subject underwent two imaging sections that include a 6 × 6-mm region centered in the fovea and a 4.5 × 4.5-mm scan centered in the optic disc. Split-spectrum amplitude decorrelation angiography (SSADA) software algorithm was used for computing a flow map of each scan. Motion Correction Technology of Optovue software was used to compensate for motion artifacts. Images with inadequate scan quality (≤6/10) were excluded from the analysis. Retinal layers were segmented between the inner limiting membrane and the retinal pigment epithelium on the basis of the optical coherence tomography structural image. Segmentation of the angiogram were checked to avoid analysis error. An en face optical coherence tomography angiogram was produced by maximum decorrelation projection of the segmented retina.

The software automatically segmented the tissue into the macula (superficial, deep, outer retina, and choriocapillaries) and optic nerve head (optic nerve head, vitreous, radial peripapillary capillary, and choroid). Macular vessel densities were measured for the superficial and deep capillary plexus. The peripapillary vessel density was measured for the radial peripapillary capillary (Fig1).

The boundaries were as follows: a slab extending from the internal limiting membrane to 10 μm above inner plexiform layer was generated for detecting the vessel density of superficial capillary plexus. A slab extending from 10 μm above inner plexiform layer to 10 μm below the outer plexiform layer for the measurement of vessel density of deep capillary plexus and a slab extending from inner limiting membrane to retinal nerve fiber layer for the measurement of peripapillary vessel density.

Peripapillary region was divided into 8 sectors (Fig 1a), designated as nasal superior, nasal inferior, inferior nasal, inferior tempo, tempo inferior, tempo superior, superior tempo and superior nasal. Foveal avascular zone area (mm²) evaluated in the superficial plexus was identified as non-flow area and measured by the built-in software that delineated the segment of the foveal avascular zone automatically. Foveal thickness was calculated from the mean central point of fovea from inner limiting membrane to retinal pigment epithelium. Foveal density 300 (FD 300) is identified as the vessel density of the annular area surrounded by 300μm outwards with the FAZ inner boundary.

Two readers (DC and ZNH) evaluated the angiographic features on optical coherence tomography angiogram. Both readers were masked to the DR status. Image quality was considered by including images having Scan Quality of at least 6/10. In patients with poor images, we repeated the scans until an image with at least fair quality could be obtained.

Statistical Analysis

Statistical analysis was performed with SPSS 19.0 software (SPSS, Inc, Chicago, IL, USA). Qualitative variables are presented as number and percentage. Quantitative variables are presented as means and standard deviations. One-way analysis of variance test was used to compare the mean values of vessel

density of superficial, deep and peripapillary area between groups. Receiver operator characteristic (ROC) curve was used to assess the relationship between the severity of DR and OCTA parameters and detect the sensitivity and specificity of the ROC curve. Criterion significance was assessed at the $P < 0.05$ level.

This study has been performed in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Guangdong General Hospital (registration number: gdrec2016232A).

Results

Demographics

A total of 170 eyes of 43 normal controls and 127 eyes of 127 diabetic patients with NPDR (mild NPDR, 43; moderate NPDR, 42; severe NPDR, 42) were assessed initially for image quality after they met inclusion and exclusion criteria described in methods. Baseline demographics of the entire cohort were comparable in all 4 groups (Table 1). Of the 170 patients, 92 (54.1%) were male and 78 (45.9%) were female. Gender and age showed no significant differences in between-groups comparison. Of the 170 selected eyes, 88 (51.8 %) were right eyes, and 82 (48.2 %) were left eyes. Patients were classified into normal group and NPDR (mild NPDR, moderate NPDR, severe NPDR) groups.

Vessel density index and RNFL thickness measurement

A series of color fundus photos and OCTA color-coded vascular maps were listed as non-proliferative diabetic retinopathy progresses were shown in *Fig 2*. Details of vessel densities in different layers of macular area, peripapillary area were illustrated in *Table 2* and *Fig 3*. Vessel densities in SCP and DCP of macular area, and peripapillary area were ($53.13\% \pm 3.03\%$, $52.07\% \pm 2.28\%$ and $53.78\% \pm 3.66\%$) in control subjects, ($50.24\% \pm 3.81\%$, $47.40\% \pm 5.02\%$ and $46.50\% \pm 6.03\%$) in mild NPDR, ($46.80\% \pm 5.23\%$; $44.39\% \pm 3.99\%$ and $44.64\% \pm 4.23\%$) in moderate NPDR, ($42.82\% \pm 5.46\%$, $42.34\% \pm 5.14\%$ and $43.16\% \pm 4.47\%$) in severe NPDR. Vessel densities in SCP and DCP of macular area as well as vessel density in peripapillary area showed significant decrease as DR progresses.

Vessel densities in different quadrants of peripapillary area and RNFL thickness were also shown in Table 2. Significant differences in between-groups comparison were found in the vessel densities at all quadrants ($p < 0.05$). In the present study, peripapillary vessel density was significantly correlated with vessel densities of SCP and DCP (*Fig.4*). In terms of RNFL thickness, significant difference was also found in comparison among groups ($p = 0.03$).

AUROC analysis

Table 3 detailed the performance of FD 300, vessel densities of SCP and DCP in macular area, and peripapillary area for identifying severity of DR based on AUROC curve. AUC in FD300, SCP and DCP of

macular area and peripapillary area and were 83.5%, 90.5%, 91.3% and 93.0%, respectively. Vessel density of DCP shows better ability to identify the severity of DR (0.913; 95% CI = 0.867–0.958; cut off value:0.75) than in FD 300, SCP of macular area and peripapillary area (*Fig. 5*).

Discussion

In the present study, as DR progressed, the reduction in vessel densities of macular area and peripapillary area as well as RNFL thickness were noted. As for peripapillary area, vessel densities from different quadrants all decreased and were significantly related to the scale of DR. In terms of AUROC analysis, vessel density of DCP in macular area showed better ability to identify the severity of NPDR when compared to vessel densities of SCP, RPC density and FD300.

The pathology of DR remained as the object of conjecture and hypotheses. Traditionally, DR has been considered as one of the microvascular diabetic complications and can generally be described as progressing through two stages, including NPDR and proliferative DR.[17] Hyperglycemia was considered to be an important factor in the etiology of DR and initiates downstream events such as basement membrane thickening, pericyte apoptosis and capillary occlusion.[18, 19] Inflammation, endoplasmic reticulum function disorders and elevated level of reactive oxygen species (ROS) are major causative factors involved in the pathogenesis of DR which can finally lead to vessel density defects in retina as well as in ONH.[20–22]

In our research, we documented the reduction in the macular vessel density related to the severity of DR stage, which is in line with most of the past published literatures such as of Agemy et al [11] and lee et al. [23] These similar results indicate that macular vessel density agreed closely with the severity of DR. We further analysis vessel densities of peripapillary area and of its different quadrants. It is noticed that no matter as a whole or in quadrants, vessel densities of peripapillary area decreased as DR progresses. To the best of our knowledge, this is the first study using OCTA to demonstrate such a decrease tendency in ONH perfusion in NPDR patients. Our results indicated that the reduced vessel density of ONH in diabetic eyes was prominent and such alteration could be another sensitive indicator for the progression of DR.

Histologically, ONH is supplied by two main source of blood flow: the superficial layers of the optic nerve head (nerve fiber layer on the surface of the optic disc) by the central retinal artery (CRA) circulation; and the deeper layers (the prelaminar, lamina cribrosa, and retrolaminar regions) by the posterior ciliary artery (PCA) circulation. [24, 25] The radial peripapillary capillaries (RPCs) are located in the inner part of the nerve fiber layer. They are parallel to the retinal ganglion cell axons and arched up steeply to supply superficial RNFL around the ONH. [26, 27] Previous Studies have indicated that when neuron activity increased locally in visual stimulus, retinal arterioles dilated to ensure adequate blood supply for actively firing neurons. [28]The regulation of blood flow was considered in response to neuroactivity. However, recent research has pointed out that retinal neurodegeneration might precede microvascular dysfunction in DR.[29–32] Many researches have suggested neuronal loss in the inner retina of DR eyes by demonstrating the reduction of nerve fiber, ganglion cell and plexiform layers using optical coherence

tomography, indicating retinal neurodegeneration is an early component of DR, which might precede visible vasculopathy. Relative Studies also have found out that RNFL thinning correlated positively with severity of DR.[33] In the present research, we also documented significant difference in average RNFL thickness in-between group comparison. RNFL thickness reduced as the severity of DR increased. Our understanding of the pathophysiology process of DR has been revolutionized by decades of research. The present study agrees strongly with the idea that DR should be more accurately defined as a neurovascular rather than a microvascular disease. Further studies are definitely required for the clarification of which one trigger the occurrence of DR.

According to the previous research, the vascular networks become more complex with multiple overlapping capillary beds away from the foveal center. It has raised the concern that OCTA may not be able to display some of the finer capillary structure, which might influence the accuracy of vessel densities[12]. The alteration of foveal avascular zone, such as capillary drop out around FAZ area, has even been identified in patients with early stage of DR[34–36]. FD 300 is identified as the vessel density of the annular area surrounded by 300µm outwards with the FAZ inner boundary. It is reasonable to consider vasculature in FD300 was organized into less layers and FD300 as another sensitive indicators for the severity of DR. Thus, we intend to use FD 300 to predict the progress of NPDR in the present study. However, AUROC analysis showed that vessel density of DCP shows better ability to identify the severity of DR than other vessel density indexes. It is still of great interest to analyze FD 300 in different fundus diseases.

Indeed, related literature has shown that the reduction of macular vessel density is in accordance with the progression of DR and other researches have been conducted to observe the morphology neovascularization of the disc.[7, 8, 37] Compared with previous researches, the present study has several strengths. Firstly, the decrease of vessel density and RNFL thickness are notable in the current research. Since the initial course of DR is still controversial now, the current study might have provided a new insight in the occurrence and development of DR in human using OCTA. Also, our results might imply that therapeutic strategies targeting signaling pathways that cause microvascular dysfunction and retinal neurodegeneration are both important in preventing the development of DR. Hence, we probably should pay equal attention to the intervention for arresting retinal vasculopathy and neurodegeneration. Secondly, patients with diabetic optic neuropathy (DON) can take place in all stages of DR. The current research revealed the reduction of RNFL thickness as DR progressed, which might be consistent with the hypothesis proposed by previous researches that [non-arteritic anterior ischemic optic neuropathy](#) (NAION) might have a devastating effect on the integrity of the optic nerve followed by RNFL loss. [38–40] Last but not least, the clinical relevance of the present research reveals that when evaluating OCTA parameters of DR patients, vessel densities of macular area and optic disc, as well as RNFL thickness should all be taken into consideration to avoid misdiagnosis and/or missed diagnosis of patients' condition.

Nevertheless, there are limitations of the current study. GCC measurement was not taken into account in the present research. Further study would be highly recommended to take this factor into consideration.

Also, we acknowledge that this was merely an extrapolation of our results, a longitudinal study should be further conducted before such conclusion could be verified.

In conclusion, our results indicated that as DR progressed, the reduction of vessel density in macular and peripapillary area as well as RNFL thickness were prominent. Vessel density of DCP in macular area showed good ability to identify the severity of NPDR. With regard to the optic nerve, microvascular insufficiency and RNFL defects were noted distinctly. OCTA might be a good tool for the pathophysiological investigation for DR since OCTA can quantify vessel density and neurodegenerative changes in subjects with diabetes.

Abbreviations

NPDR: non-proliferative diabetic retinopathy; OCTA: optical coherence tomography angiography; SCP: superficial capillary plexus, DCP: deep capillary plexus; RNFL: retinal nerve fiber layer; FA: fluorescein angiography; ONH: optic nerve head; T2DM: type 2 diabetes mellitus; BCVA: best corrected visual acuity; SSADA: Split-spectrum amplitude decorrelation angiography; RPC: radial peripapillary capillary; NS: nasal superior, NI: nasal inferior, IN: inferior nasal, IT: inferior tempo, TI: tempo inferior, TS: tempo superior; ST: superior tempo; SN: superior nasal; ILM: inner limiting membrane; FAZ: foveal avascular zone area; ROS: reactive oxygen species; CRA: central retinal artery; PCA: posterior ciliary artery; DON: diabetic optic neuropathy;

Declarations

Ethics approval and consent to participate

This study has been performed in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Guangdong General Hospital (registration number: gdrec2016232A).

Consent to publish

Written informed consents for publication of the clinical details and accompanying images were obtained from the patients. A copy of the written consents is available for review by the Editor of this journal.

Availability of data and materials

All data generated or analyzed of this study are included in this article. Raw and processed data during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declared **no** conflicts of interest to this work

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Authors' contributions

Conception and design of the study: LZ, DC, HHY, XHY;

Acquisition of data: DWY, YKZ, XNZ, BYL, QWW.

Analysis and interpretation of data: DWY, ZNH, YKZ

Drafting of the manuscript: DWY, XNZ

Critically revision of the manuscript: LZ, DC, HHY

Provision of materials, patients or resources: LZ, HHY, ZNH

All authors read and approved the final version to be published

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Tables

Table 1 Demographic and ocular characteristics of the participants in four groups.

	Normal	Mild NPDR	Moderate NPDR	Severe NPDR	P value
Number of eyes	43	43	42	42	0.218
Gender (female: male)	22:21	24:19	24:18	22:20	0.391
Left/right eye	23:20	21:22	21:21	23:19	0.323
Age(years)	42.6±8.2	43.1±7.1	42.4±10.1	44.0±9.3	0.107

Table 2 Macular perfusion and peripapillary perfusion and RNFL thickness of subjects observed

	Normal	Mild NPDR	Moderate NPDR	Severe NPDR	p value
Macular perfusion of SCP	53.13±3.03	50.24±3.81	46.80±5.23	42.82±5.46	p <0.001
Macular perfusion of DCP	52.07±2.28	47.40±5.02	44.39±3.99	42.34±5.14	p <0.001
FD300	55.04±4.89	49.09±4.04	48.05±5.35	45.91±7.92	p <0.001
Peripapillary perfusion	53.78±3.66	46.50±6.03	44.64±4.23	43.16±4.47	p <0.001
Nasal Superior	48.07±3.01	46.18±4.66	43.23±5.20	40.51±6.57	p <0.001
Superior Nasal	51.92±3.36	48.10±5.84	46.65±5.78	40.40±8.28	p <0.001
Nasal Inferior	48.81±2.81	44.89±4.73	42.46±5.27	38.42±5.58	p <0.001
Inferior Nasal	51.85±3.93	49.93±4.89	46.99±5.34	40.41±8.05	p <0.001
Inferior Tempo	57.55±3.02	56.81±4.58	53.13±4.79	47.63±9.72	p <0.001
Temporal Inferior	54.20±3.44	51.32±3.96	49.76±5.63	45.67±7.62	p <0.001
Tempo Superior	57.45±2.88	54.70±4.32	51.77±5.41	46.95±8.09	p <0.001
Superior Tempo	55.94±3.72	53.20±4.60	51.64±5.60	46.43±9.94	p <0.001
Peripapillary RNFL thickness (µm)	128.86±11.32	124±13.97	121.02±20.86	118.60±21.91	P =0.03

Vessel density in SCP, DCP and peripapillary area were reported as percentage.

NPDR: Non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy

SCP: superficial capillary plexus; DCP: deep capillary plexus

Table 3 Performance parameters for perfusion index for identifying severity of DR

	AUROC (95% CI)	Sensitivity (%)	Specificity (%)	Youden Index
VD in SCP in macular area	0.913(0.867-0.958)	83.1	85.2	0.683
VD in DCP in macular area	0.930(0.886-0.975)	88.1	86.9	0.750
VD in peripapillary area	0.905(0.860-0.950)	83.3	83.6	0.669
FD 300	0.835(0.760-0.910)	82.7	59.2	0.419

AUROC: Area under receiver-operating characteristic curve

VD: vessel density

Figures

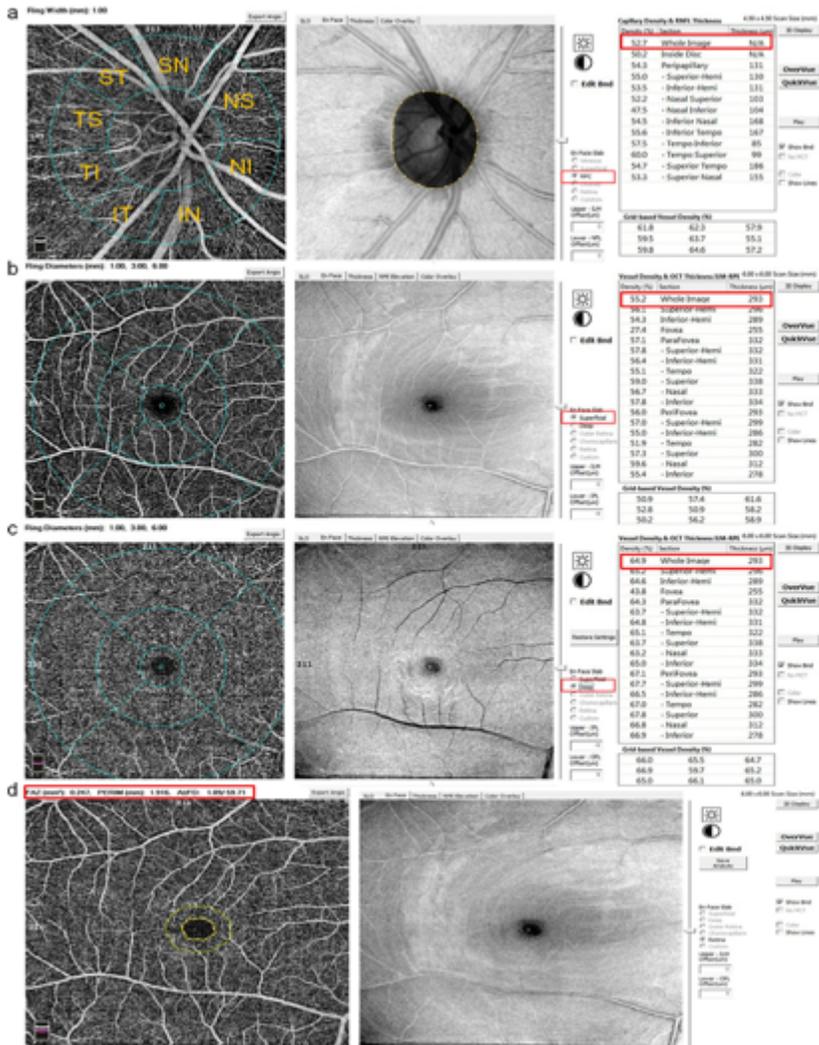


Figure 1

Example images of vessel density and RNFL measurement in a control subject (a. radial peripapillary capillaries; b. superficial capillary plexus; c. deep capillary plexus; d. quantification of FD300). Vessel densities and RNFL thickness were calculated automatically (red box).

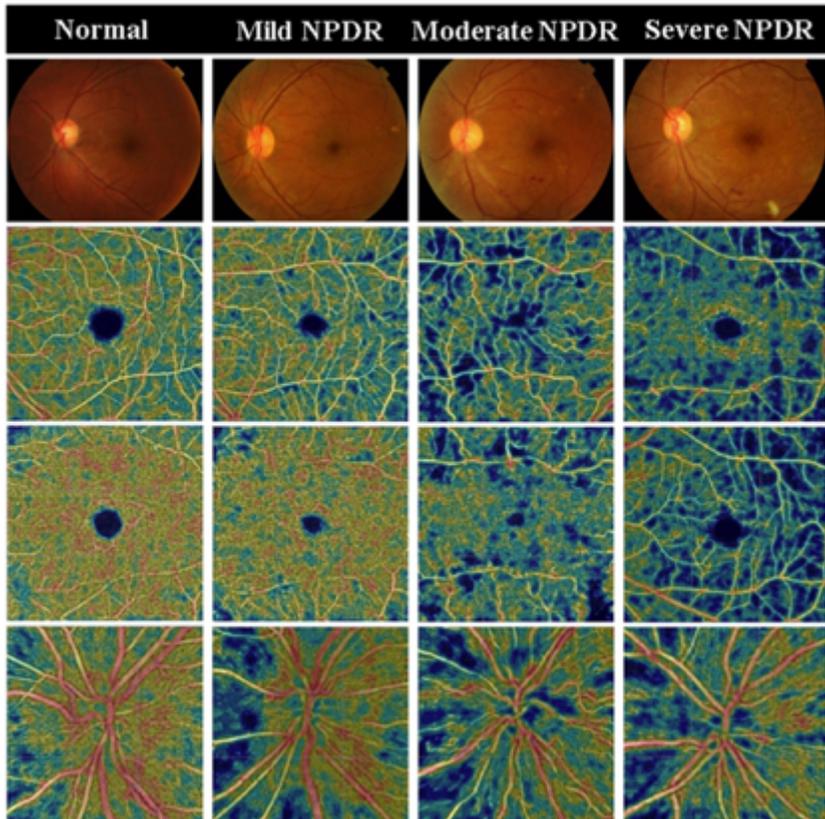


Figure 2

A series of color fundus photos (top row), SSADA OCTA color-coded vascular perfusion maps (second to the last row) demonstrating the perfusion density changes seen as non-proliferative diabetic retinopathy progresses. From left to right, we start with a normal eye and progress to mild NPDR, moderate NPDR, and finally severe NPDR.

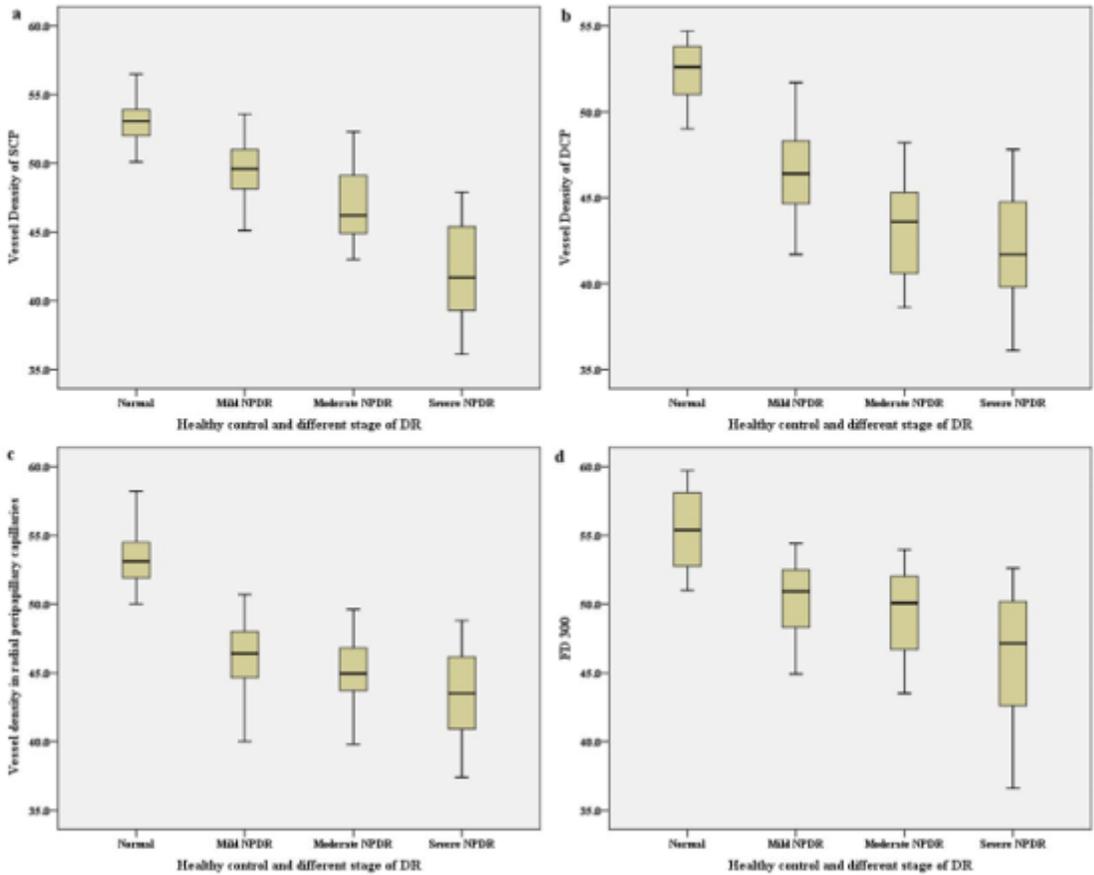


Figure 3

Box plots demonstrating the vessel density changes with normal control and different stage of DR.

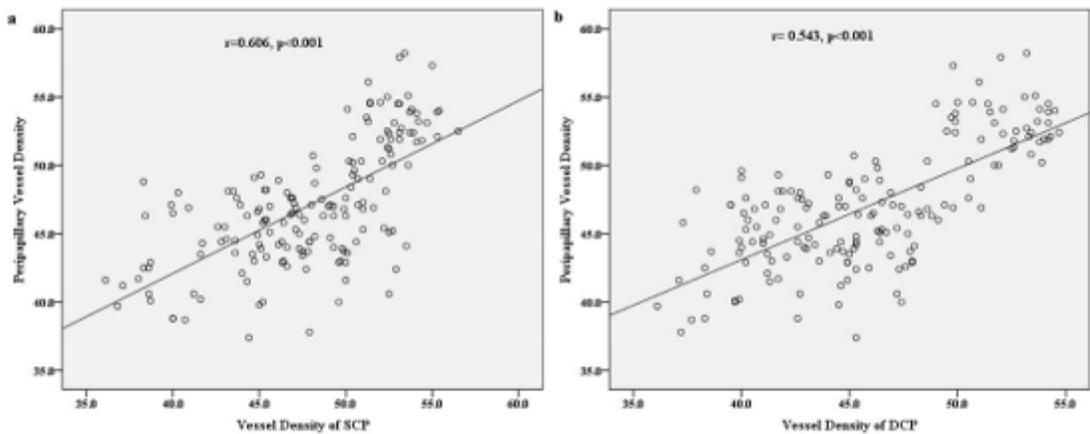


Figure 4

Correlation analyses between peripapillary vessel density and vessel density of SCP and DCP in macular area. Peripapillary vessel density was significantly correlated with vessel density of SCP (a) ($r=0.606$, $p<0.001$) and vessel density of DCP (d) ($r=0.543$, $p<0.001$)

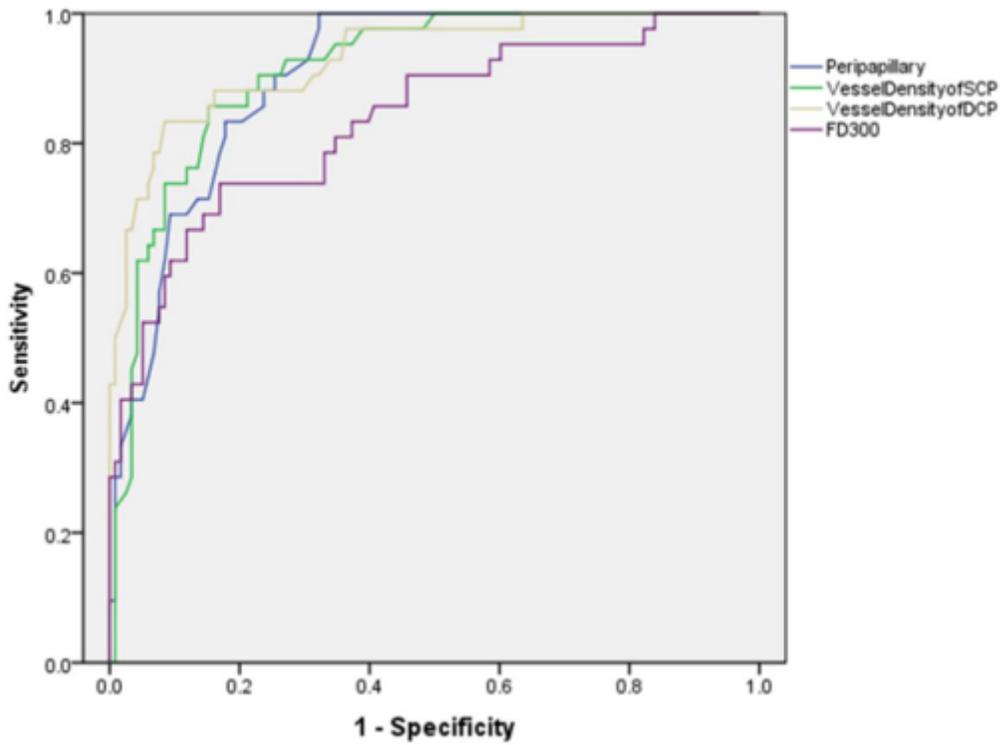


Figure 5

Receiver-operating characteristic (ROC) curves for SCP and DCP of macular area, and peripapillary area and FD 300 for detecting stage of DR.