Synchronous evaluation of early neurovascular changes in macula and optic nerve head in early diabetic retinopathy using combined optical coherence tomography angiography parameters

Xin Wen
Sun Yat-Sen Memorial Hospital, Sun Yat-sen University
Zijing Li
Sun Yat-sen Memorial Hospital
Jiaqi Liang
Sun Yat-sen Memorial Hospital
Jianhui Xiao
Sun Yat-sen Memorial Hospital
Yichi Zhang
Sun Yat-sen Memorial Hospital
Xiang Gao
Sun Yat-sen Memorial Hospital
Yunru Liao
Sun Yat-sen Memorial Hospital
Yuqing Lan (lanyq@mail.sysu.edu.cn)

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Abstract

Background This study aim to evaluate neurovascular changes in both macula and optic nerve head (ONH) in early stages of diabetic retinopathy (DR) using combined quantitative optical coherence tomography angiography (OCTA) parameters.

Methods We studied 194 right eyes from 64 type 2 diabetic patients without DR (DM), 64 diabetic patients with nonproliferative diabetic retinopathy (NPDR) and 64 age-matched healthy controls. OCTA parameters were analyzed using binary logistic regression models and receiver operating characteristic (ROC) curves.

Results There was significant reduction of vessel density (VD) in both macula and ONH comparing DM patients with and without NPDR to controls. The area under the curve (AUC) of the ROC curve for NPDR versus control was 0.963 (p <0.001), with sensitivity of 93.8% and specificity of 90.6%. The ROC curves for NPDR patients versus NoDR patients (including DM and control groups) exhibited an AUC of 0.923 (p <0.001), with sensitivity of 90.6% and specificity of 82.8%. The thickness of the retinal nerve fiber layer (RNFL) in both macula and ONH was not significantly different among these three groups.

Conclusions VD in both macula and ONH was simultaneously decreased prior to RNFL thinning in DM patients through the course proceeding from preclinical DR to NPDR. Combined analysis of macula and ONH parameters was an comprehensive and accurate OCTA metric to distinguish NPDR patients from healthy controls and DM patients without DR.

Background

Diabetic retinopathy (DR) is a leading cause of acquired blindness in working-age adults worldwide[1, 2]. DR is classically thought to be a microvascular disorder that is likely to occur in the posterior pole. Several lines of evidence show that neurodegeneration, including decreases in peripapillary retinal nerve fiber layer (RNFL) thickness is present before clinical DR in patients of diabetes mellitus (DM)[3]. A recent study showed positive correlations between peripapillary blood flow and RNFL thickness around the optic nerve head(ONH) [4]. However, these studies analyze the vessel density (VD) and RNFL of either the macula or ONH separately and therefore cannot conclude whether the DR changes of macula and ONH are synchronized pathological processes or are separate pathological changes. They cannot determine whether retinal microvascular disorders occur prior to retinal neural complications or vice versa from a comprehensive view.

Recently, the development and application of optical coherence tomography angiography (OCTA) has enabled the blood flow in the retina and ONH to be quantitatively visualized in a non-invasive way[5]. By contrast with other fundus imaging techniques such as fundus fluorescein angiography (FFA), OCTA has the advantage of quantitatively visualizing the vessel network and retinal nerve fiber layer-by-layer. Repeatability is found to be satisfactory for measuring the parameters of macula and ONH[6], and repeated measurements using the same device are credible[7, 8]. A variety of OCTA parameters concerning the macula or ONH have been explored, including VD of the superficial capillary plexus (SCP) and the deep capillary plexus (DCP) layer, foveal avascular zone (FAZ) parameters and RNFL thickness parameters. The

results are controversial across various reports. Several studies suggest that OCTA parameters vary with the stage of DR[9–11]. Some suggest that vessel parameters in the SCP are significantly different among the various stages of DR[11] while others find that the vessel parameters in the DCP, not in the SCP, are significantly different between eyes of nonproliferative DR (NPDR) and proliferative DR (PDR)[12]. There are also reports indicating that the VD in the ONH[13] as well as the thickness of RNFL[4] are significantly different between the DM and normal subjects.

In these studies, the OCTA parameters of macula or ONH were studied individually. Few studies produced integrated analyses of the dominating parameters to eliminate the interactions among parameters. Therefore, the aim of this study was to synchronously study the parameters of macula and ONH and to identify the neuropathy and vasculopathy in early DR from a comprehensive perspective. Hopefully, such a study would be helpful to abtain a better understanding of pathological process in DR and the clinical application of identifying early signs of DR.

Methods

Study design and subjects

This cross-sectional case-control study recruited 64 type 2 DM (T2DM) patients without any DR (DM group), 64 T2DM patients with NPDR (NPDR group), and 64 healthy control subjects (control group) who presented between October 2017 and February 2019. Patients in these three groups were age-matched. The study was approved by the research ethics committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University, and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants. The data from the right eyes of all patients were collected and analyzed. Patients with diagnosis of T2DM, disease duration≥1 year were included. Exclusion criteria included: ocular disease with severe opacity of refractive media that would influence fundus examination and any other ocular disease that would affect ocular circulation, including glaucoma, myopia with refractive error greater than 3 diopters, age-related macular degeneration, macular edema and retinal artery/vein occlusion. All patients were examined with best-corrected visual acuity (BCVA) (LogMAR visual acuity), intraocular pressure (IOP), slit-lamp biomicroscopy, and, after pharmacologically dilating pupils, indirect ophthalmoscopy, color fundus photographs, OCTA and FFA (only in patients with NPDR). The results were reviewed by two experienced ophthalmologists (XW and ZJL) independently and the diagnoses were made according to the proposed international diabetic retinopathy severity scale[14].

OCTA image acquisition

The OCTA images were acquired using an RTVue XR Avanti spectral domain OCT device (Optovue, Inc., Fremont, CA, USA) with a speed of 70,000 A-scans/second and a wavelength of 840 nm. A 3 mm × 3 mm area of macula and a 4.5 mm × 4.5 mm area of ONH were scanned. A split-spectrum amplitude-decorrelation angiography (SSADA) algorithm was used to detect erythrocyte movement in OCTA. Retinal vascular networks and the nerve fibers of macula and ONH were analyzed. OCTA images without

significant motion or shadow artifacts and with scan quality ≥ 6 were included. OCTA images with poor quality or large motion artifacts or segmentation errors were excluded.

The parameters of the macula and ONH were automatically measured and analyzed using prototype AngioVue software 2.0 without any attempts at manual alteration. The parameters of choroidal capillary were not included for the requirement of manual measurement. Macula-associated parameters included whole VD of SCP and DCP, FAZ area, retinal thickness of the superficial slab started from internal limiting membrane (ILM) to retinal pigment epithelium (RPE) and the deep slab started from RPE to Bruch's membrane (BRM). VD was defined as the percentage of the area that was occupied by blood vessels. The area of vessels was calculated using the software by extracting a binary image from the grayscale OCTA image, and then calculating the percentage of pixels occupied by blood vessels in the defined region. The SCP slab was segmented from the ILM to the inner plexiform layer (IPL). The DCP slab was segmented from 15μm posterior to the IPL to the outer plexiform layer (OPL). FAZ perimeter (PERIM), acircularity index (AI), foveal vessel density in a 300-μm region around FAZ (FD), foveal VD, parafoveal VD and perifoveal VD in the SCP and DCP were not included because the high correlation between any two of the above parameters would influence the results of the statistical model. ONH-associated parameters included VD of the whole 4.5 mm × 4.5 mm area, inside the ONH, peripapillary area as well as the average RNFL thickness of the entire peripapillary area. The peripapillary area was defined as a 1.0 mm-wide round annulus extending from the 2.0-mm circle of ONH.

Statistical analysis

The statistical analyses were performed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA). One-way ANOVA with further Bonferroni analysis were performed to analyze the OCTA variables of patients in the control, DM and NPDR groups. For variables that were statistically significant, a multivariable binary logistic regression model using backward elimination for the nonsignificant variables was further conducted, including models with comparison among eyes of controls, DM and NPDR groups as well as a model comparing eyes of NoDR (including eyes of both control and DM groups) with those of NPDR. The receiver operating characteristic (ROC) curves were generated based on the binary logistic regression models. Area under the curve (AUC), sensitivity, and specificity were calculated in the ROC curve. A value of $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics

One hundred and ninety-two eyes of 192 patients were enrolled. The mean age was 53.94 ± 9.19 years old (range, 29 to 69 years old). Eyes of the NPDR group included 20 eyes with mild NPDR, 26 eyes with moderate NPDR, and 18 eyes with severe NPDR. All OCTA images were taken prior to FFA examination, panretinal photocoagulation or anti-VEGF injection treatment. There were no significant differences among control, DM and NPDR groups with respect to gender (male vs female: 36:28 vs 38:26 vs 40:24,

respectively), IOP (16.47 ± 2.39 mmHg vs 16.08 ± 2.65 mmHg vs 15.64 ± 2.45 mmHg, respectively), presence of hypertension (25.00% vs 28.13% vs 28.13%, respectively) or glycosylated hemoglobin (HbA1c) (10.03 ± 7.85% in DM group vs 8.6 ± 2.35% in NPDR group, $p = 0.186$). The diabetes duration was significantly longer in NPDR group versus DM group (8.39 ± 5.78 years vs 6.03 ± 5.39 years, $p = 0.018$). LogMAR BCVA was significantly better in the control and DM groups than in NPDR group (p 0.001 and p 0.001, respectively). There were also significant differences in LogMAR BCVA between NoDR and NPDR groups (−0.05 ± 0.08 vs 0.04 ± 0.10, p 0.001) (Table 1).

OCTA findings

Univariate analysis and multivariable logistic regression of the OCTA parameters showed that whole VD in the DCP and ONH were significantly different between control group and DM group. ROC curves for these variables using the combined parameter model were generated. The AUC was 0.798 ($p < 0.001$) with sensitivity of 82.8% and specificity of 67.2% (Fig 1A). The statistical model also showed that parameters such as whole VD of SCP and inside disc VD were significantly different between the DM and NPDR groups. The AUC was 0.860 ($p < 0.001$) with sensitivity of 85.9% and specificity of 70.3% (Fig 1B). Several parameters associated with the macula and ONH were significantly different when comparing the control group to the NPDR group, including whole VD in SCP and DCP, whole VD in ONH and Peripapillary VD. The same approach of ROC was carried out. We found an AUC of 0.963 ($p < 0.001$) with sensitivity of 93.8% and specificity of 90.6% (Fig 1C). When comparing the NoDR group with NPDR group, the differences were significant in whole VD of SCP and DCP, whole VD of ONH, inside disc VD and peripapillary VD. The ROC curve showed an AUC of 0.923 ($p < 0.001$) with sensitivity and specificity of 90.6% and 82.8%, respectively (Fig 1D). There was no significant difference in average RNFL thickness of both ONH-associated and macula-associated parameters among these groups (Table 2, Table 3 Fig 2).

Discussion

DR has been customarily considered as a retinal microvascular disease. Recently, several lines of evidence suggested that neurodegenerative changes occur in early diabetes. Apoptosis of retinal ganglion cells was observed in the diabetic retina[15]. Corresponding clinical evidence showed subtle changes in color perception and contrast sensitivity and significant thinning of RNFL in diabetic eyes without DR[16, 17]. Recently, the relationship between microvascular changes and neural changes was also analyzed. A study reported that ONH perfusion in diabetic patients without DR was significantly lower than that of controls[4, 18], and concluded that reduced ONH perfusion preceded peripapillary RNFL thinning. However, they did not include NPDR objects; therefore, they could not answer the question regarding whether microvascular changes or neural impairments occur first in the early onset of DR. Our study comprehensively analyzed the retinal vessels of macula and ONH together with RNFL changes and demonstrated that vascular changes, specifically the VD, occurred prior to detectable RNFL changes in both the macula and ONH.

Another interesting issue that has been little studied is the relationship between OCTA parameters of macula and ONH. To the best of our knowledge, this was the first study to analyze OCTA parameters in both

the macula and ONH in preclinical DR and NPDR. We found that both VD in macula and ONH were simultaneously reduced prior to retinal neural changes and clinically-visible retinopathy. This could be explained anatomically as follows: the superficial layers of ONH deriving from the central retinal artery (CRA) circulation[19, 20] and the radial peripapillary capillaries (RPCs), the density of which we detected using OCTA, might derive from the arterioles that were parallel to retinal ganglion cell axons [21] and were clustered radially to supply superficial RNFL around the ONH[22, 23]. Previous histological reports also showed that RPCs were derived from the superficial vascular plexuses that supplied the macular area and ascended to the nerve fiber layer[24, 25]. Therefore, several mechanisms of DM may cause dysregulation of cellular physiology and may damage vascular cells, possibly leading to capillary occlusion and decreased VD in both ONH and macula.

VD could be influenced by age and gender[26, 27]. In this study, the factor of age was eliminated and the factor of gender was not significantly different. The correlation between VD and glycosylated hemoglobin (HbA1c) is controversial[11, 26]. In this study, HbA1c was not significantly different between eyes with and without DR. VD examination in OCTA by automated method showed high reproducibility and reliability[7]. It is reasonable that this set of VD parameters may be able not only to detect diabetic eyes at a higher risk of NPDR but also to screen for NPDR even before systemic diagnosis is made[28]. Some studies found that VD in DCP on OCTA, not in SCP or choriocapillaris, was lower in diabetic patients without DR compared to nondiabetic individuals[29–31]. Other studies showed that not only VD in the DCP but also VD in the SCP were different between diabetic patients and healthy controls[10, 32, 33]. In this study, which took the parameters of both macula and ONH into consideration, both SCP and DCP were significantly different among DM patients with or without DR and healthy controls. Consistent with the anatomical fact that DR could affect retinal vessels in both SCP and DCP, such result indicated that this statistical model could reduce the chance of drawing one-side conclusions and could analyze the VD changes from a more comprehensive view. Consistent with results of other studies[7, 12], which showed a negative correlation between the logMAR BCVA and the VD in the SCP and DCP, our result also showed higher logMAR BCVA in NPDR group in which the VD of SCP and DCP were lower.

In this study, we identified a small set of selected OCTA parameters that accurately distinguished eyes of normal control and DM patients from those with NPDR. Compared to individual parameters with ROC ranging from 0.472 to 0.893 [26], the combined model had the advantage of higher sensitivity/specificity as well as accuracy, making it a useful tool for analyzing the neurovascular changes of DR[34]. The current study demonstrated that combining a small set of selected macula-associated OCTA parameters, the accuracy of which is similar to our results, improved the overall diagnostic efficacy for discriminating eyes with NPDR from those with PDR[34]. To the best of our knowledge, this is the first study to analyze in an integrated fashion the widely-studied OCTA parameters of both the macula and the ONH in patients with preclinical DR. Furthermore, our results demonstrated a minimal set of VD parameters in the macula and ONH with significant difference and favorable sensitivity and specificity to detect the very early changes in preclinical DR. Such set of OCTA parameters was with high sensitivity and specificity to differentiate diabetic patients especially those with NPDR from healthy controls and DM patients without any DR. Interestingly, many variables that were significant in the univariate analysis of recent study were not

significant in the final model[11, 35]. This suggested that individual analyses could neglect the interaction among the parameters. It also suggested that the combined models could highlight the dominating parameters affecting the pathogenetic process of DR in early stages and set up a reliable evaluation system of early vasculopathy in DM.

Based on our findings, we suggested a new perspective for the pathophysiological investigation of DR. Because microvascular alterations might occur earlier than neurodegenerative changes in both macula and ONH in the early onset of DR, therapeutic strategies should target the signaling pathways that could remedy microvascular dysfunction for the purpose of preventing the development of DR, and the target area should include both the macula and the ONH.

One limitation of this study was that it was an observational cross-sectional study, possibly missing a definite conclusion. Another limitation was that automatic segmentation was applied to analyze the parameters; therefore, the possibility of segmentation errors could not be ruled out. Furthermore, we did not include all the possible parameters of macula and ONH as well as parameters of choroidal capillary, possibly leading to deficiencies of parameters with significant differences. Further longitudinal study with expanded parameters are needed to resolve these issues.

Conclusions

Our results indicated that reduction of VD simultaneously occurred in both the macula and ONH prior to RNFL thinning in the early stage of DM. We provided a statistical model combining the parameters of both macula and ONH to identify early vasculopathy of DM and to differentiate NPDR patients from healthy controls and DM patients without any DR.

Abbreviations

ONH: optic nerve head; DR: diabetic retinopathy; OCTA: optical coherence tomography angiography; NPDR: nonproliferative diabetic retinopathy; ROC: receiver operating characteristic; VD: vessel density; AUC: area under the curve; RNFL: retinal nerve fiber layer; FFA: fundus fluorescein angiography; DCP: deep capillary plexus; FAZ: foveal avascular zone; PDR: proliferative DR; T2DM: type 2 DM; BCVA: best-corrected visual acuity; IOP: intraocular pressure; ILM: internal limiting membrane; RPE: retinal pigment epithelium; IPL: inner plexiform layer; OPL: outer plexiform layer; PERIM: FAZ perimeter; AI: acircularity index; FD: foveal vessel density in a 300-μm region around FAZ; CRA: central retinal artery; RPCs: radial peripapillary capillaries; HbA1c: glycosylated hemoglobin

Declarations

Acknowledgements

Not applicable.

Authours' contributions

Study design: XW and YL(Yuqing Lan); Data acquisition: XG and YL(Yunru Liao); Analysis and interpretation of data: ZL and JL; Write the manuscript: XW. Revise the manuscript: JX and YZ. All authors have read and approved the content and agree to submit for publication in the journal.

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Availability of data and materials

The datasets used and/or analysed during the current study were available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study had been approved by the research ethics committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University and was in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication

Written informed consent for publication had been obtained from participants whose fundus images were present in Fig. 2.

Competing interests

The authors declared that they had no competing interests.

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Tables

Table 1 Demographic characteristics of control, NoDR and NPDR subjects

Characteristics	Control	DM	NPDR	*p* value			
				Control vs DM	DM vs NPDR	Control vs NPDR	NoDR vs NPDR
Male:female(n)	36□28	38□26	40□24	0.720	0.717	0.472	0.533
Duration of DM(year)	NA	6.03±5.39	8.39±5.78	NA	0.018*	NA	NA
HbA1c(%)	NA	10.03±7.85	8.6±2.35	NA	0.186	NA	NA
(mmol/mol)		(86±62)	(70±9)				
logMAR BCVA	-0.06±0.08	-0.03±0.07	0.04±0.10	0.084	□0.001*	□0.001*	□0.001*
IOP(mmHg)	16.47±2.39	16.08±2.65	15.64±2.45	1.000	0.971	0.188	0.235
Hypertension(n,%)	16(25.00%)	18(28.13%)	18(28.13%)	0.689	1.000	0.689	0.818

DM, diabetic mellitus; BCVA, best-corrected visual acuity; IOP, intraocular pressure. *represented *p*□0.05.

Table 2 One-way ANOVA with Bonferroni analysis of OCTA parameters among the control, DM and NPDR groups

Characteristics	Control	DM	NPDR	*p* value			
				Control vs DM	DM vs NPDR	Control vs NPDR	NoDR vs NPDR
Macula associated parameters							
Whole VD in SCP(%)	49.58±2.79	47.58±2.22	43.79±3.87	0.001*	<0.001*	<0.001*	<0.001*
Whole VD in DCP(%)	50.79±4.31	46.86±3.02	44.04±4.16	<0.001*	<0.001*	<0.001*	<0.001*
FAZ(mm^2)	0.32±0.11	0.34±0.11	0.36±0.12	0.934	0.667	0.079	0.047
ONH associated parameters							
Whole VD(%)	56.30±2.36	54.67±2.02	53.74±2.12	<0.001*	0.048	<0.001*	<0.001*
Inside disc VD(%)	60.80±3.51	59.69±3.68	56.57±2.91	0.195	<0.001*	<0.001*	<0.001*
Peripapillary VD(%)	58.34±3.12	57.27±2.68	56.87±3.36	0.144	1.000	0.021*	0.049*
Thickness of RNFL							
Peripapillary(μm)	112.43±10.64	110.62±16.30	108.68±14.74	1.000	1.000	0.401	0.188
3mm×3mm area of macula(ILM-RPE)(μm)	307.39±14.24	308.77±13.96	307.17±19.08	1.000	1.000	1.000	0.711
3mm×3mm area of macula(RPE-BRM)(μm)	7.29±1.53	7.14±1.80	6.72±1.64	1.000	0.481	0.171	0.056

DM, diabetes mellitus; NPDR, nonproliferative diabetic retinopathy;NoDR, no DR; VD, vessel density; SCP, superficial capillary plexus; DCP, deep capillary plexus; FAZ, foveal avascular zone; ILM, internal limiting

membrane; RPE, Retinal pigment epithelium; BRM, Bruch’s membrane; *represented p□0.05.

Table 3 Multivariable logistic regression model comparing control, DM and NPDR groups

Parameters	Odds Ratio,OR	95% Wald Confidence Limits OR		*p* value	AUC, Sensitivity and Specificity
		Lower Limit	Upper Limit		
Control vs DM					
Whole VD in SCP(%)	0.880	0.725	1.068	0.195	AUC=0.798
Whole VD in DCP(%)	0.770	0.661	0.898	0.001*	Sensitivity=0.828
Whole VD of ONH(%)	0.812	0.671	0.983	0.033*	Specificity=0.672
DM vs NPDR					
Whole VD in SCP(%)	0.689	0.573	0.829	0.001*	AUC=0.860
Whole VD in DCP(%)	0.911	0.798	1.039	0.164	Sensitivity=0.859
Whole VD of ONH(%)	0.937	0.739	1.189	0.594	Specificity=0.703
Inside disc VD(%)	0.780	0.665	0.915	0.002*	
Control vs NPDR					
Whole VD in SCP(%)	0.612	0.463	0.809	0.001*	AUC=0.963
Whole VD in DCP(%)	0.715	0.561	0.912	0.007*	Sensitivity=0.938
Whole VD of ONH(%)	0.419	0.233	0.752	0.004*	Specificity=0.906
Inside disc VD(%)	0.790	0.586	1.064	0.121	
Peripapillary VD(%)	1.900	1.257	2.873	0.002*	
NoDR vs NPDR					
Whole VD in SCP(%)	0.657	0.550	0.784	0.001*	AUC=0.923
Whole VD in DCP(%)	0.849	0.744	0.970	0.016*	Sensitivity=0.906
FAZ	7.985	0.193	330.205	0.274	Specificity=0.828
Whole VD of	0.618	0.444	0.861	0.004*	

ONH(%)				
Inside disc VD(%)	0.768	0.650	0.908	0.002*
Peripapillary VD□%□	1.497	1.186	1.890	0.001*

DM, diabetes mellitus; NPDR, nonproliferative diabetic retinopathy;NoDR, no DR; VD, vessel density; SCP, superficial capillary plexus; DCP, deep capillary plexus; FAZ, foveal avascular zone; ONH, optic nerve head; AUC, area under the curve; *represented p□0.05.

Figures

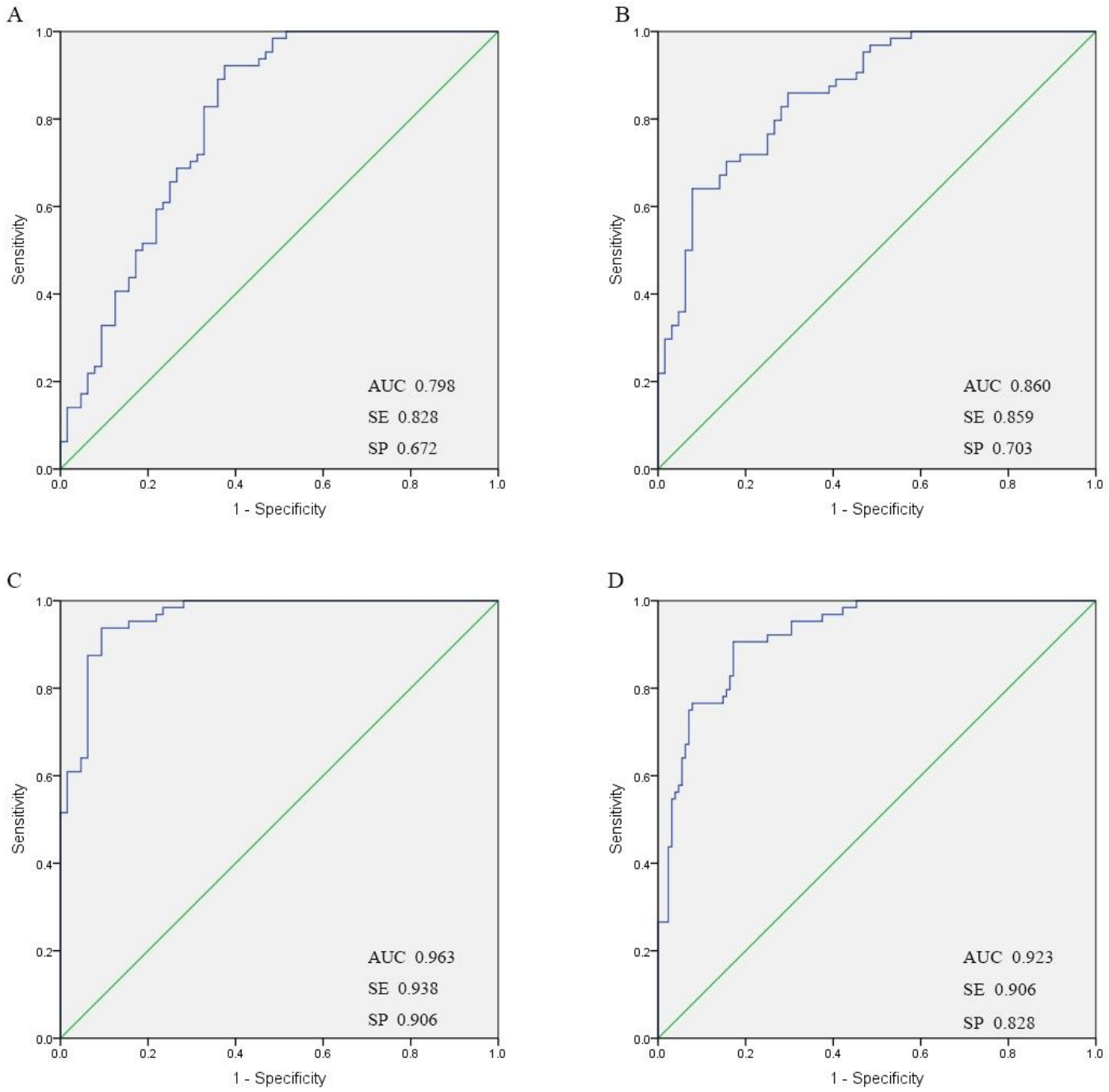

Figure 1

Receiver operating characteristic (ROC) curves for the binary regression models of combined parameters. Green lines represented reference lines.

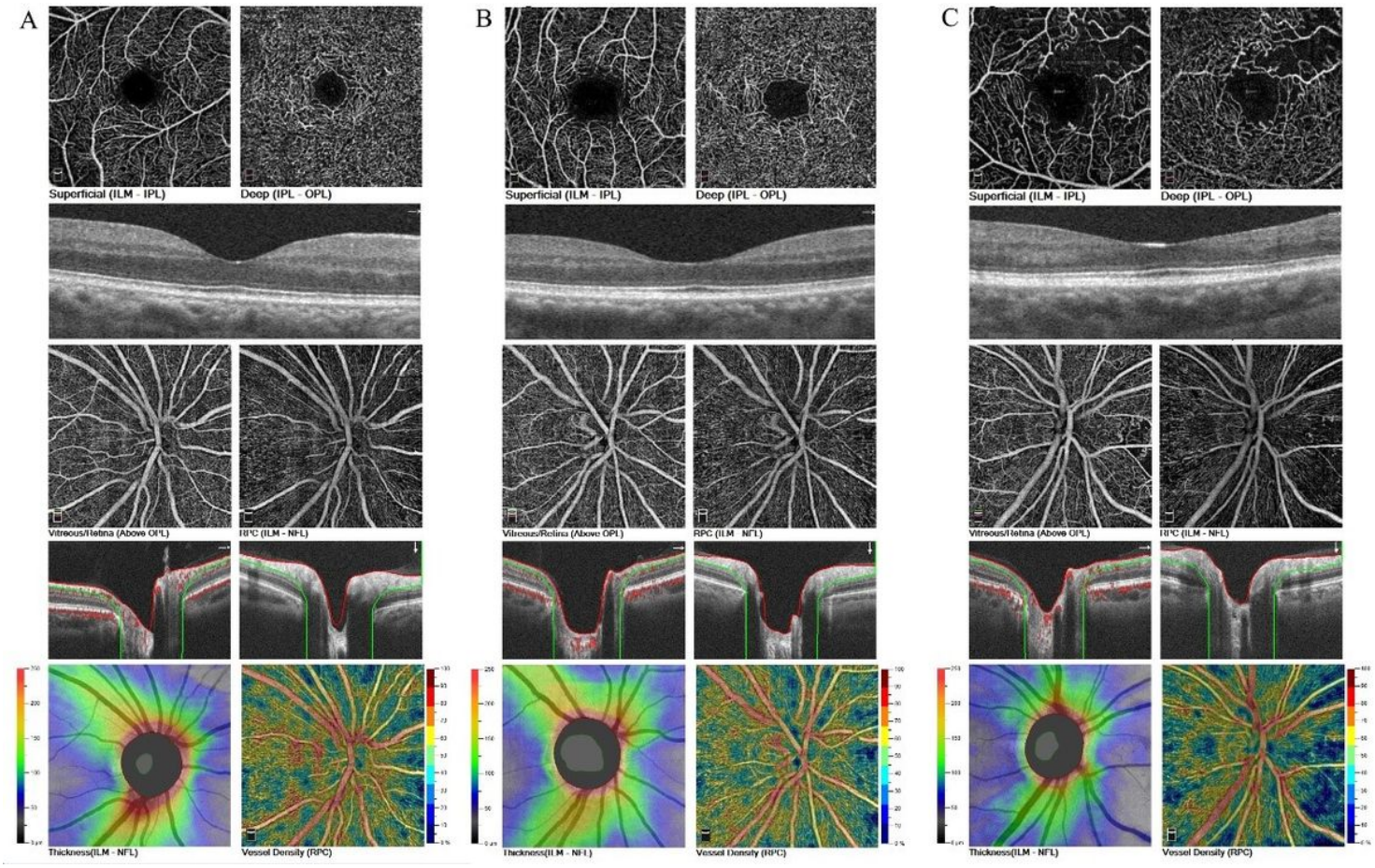

Figure 2

VD and RNFL thickness in macula and ONH of healthy control, NoDR and NPDR groups. A. The right eye of a 50 year-old female control. B. The right eye of a 50 year-old female patient with NoDR. C. The right eye of a 50 year-old male patient with NPDR. Gradually reduction of VD was observed in both macula and ONH when proceeding from normal fundus to NPDR. RNFL thickness was also decreased in these three eyes. However, it was not statistically significant among the healthy controls, NoDR and NPDR groups.