PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Changes in Peripapillary Microvasculature and Retinal Nerve Fiber Layer in Diabetes and Diabetic Retinopathy using Optical Coherence Tomographic Angiography: A Community-based, Cross-sectional Study

Authors

Liu, Jiahui; Kang, Dan; Xu, Zhiyi; Xian, Qianhong; Chen, Shuhui; Zhao, Shulun; Li, Jiali; Huang, Xuewen; Wang, Wei; Huang, Wenyong; Chen, Minyu; Wang, Langhua

VERSION 1 - REVIEW

Reviewer	**1**
Name	**van der Heide , Frank**
Affiliation	**Maastricht University, Internal Medicine**
Date	**20-Oct-2023**
COI	**none**

This population-based study among individuals with type 2 diabetes evaluated the association of stage of DR with OCTA measures (vascular density, vascular length density, vessel diameter index) and OCT measures (pRNFL), using data on 1,325 individuals with diabetes (n=210 with diabetes retinopathy and n=1,115 without diabetic retinopathy).

A major strength of the study is the large sample size.

A limitation of the study is that the authors did not evaluate interaction by age or sex. Further, there are several instances in which English language could be improved (although the English language is not bad in general).

Abstract

1. Methods section: Please add which confounders were used to adjust for in multivariable analyses.

2. Page 4 Line 57-59: The authors state that monitoring changes might be promising. This should be changed as no changes over time were studied in this study. Please use the word differences instead.

3. Page 5 Strengths (line 27,28): community based design is not a limitation, please remove this comment here.

Introduction

4. pg 6-7 Moreover, the peripapillary OCTA RNFL association during DR development and progression has not been fully addressed.

Can you specify the literature in a more detailed way? What has exactly been looked at and what has not?

5. pg 7 lines 15-16 'changes' – cross-sectional data were used so 'changes' were not measured, only 'differences', please rephrase.

Methods

6. Page 8 lines 40-41, please explain abbreviations BCVA, SE

7. Page 9. Please explain in the 'statistical analysis' section which confounders were included in the models. Please also add some additional analyses for the supplemental material in which you evaluate the impact of some potential confounders other than age, sex on the associations (e.g. evaluating a model that contains duration of diabetes, HbA1C, body mass index, systolic blood pressure, and total cholesterol) and a model that contains axial length, intraocular pressure and OCTA signal strength intensity). The eye variables may not be potential confounders as although these variables are associated with OCT/OCTa features, they may not be associated with diabetes. Adjust for these variables may thus be overadjustment.

8. Page 9 Please test whether associations differ between men and women by testing for interaction (include an interaction of e.g. sex*VD in the model and evaluate whether the P-value of this interaction term is <0.05. And in the case of interaction show stratified analyses)

Results

9. Page 9 Please add a flowchart (can be put in main manuscript or supplement).

10. Please clarify de abbreviation LT on page 11 lines7-9.

11. Page 11 paragraph starting on lines13-15. Please specify that this paragraph refers to univariable analyses

12. Page 11 paragraph starting on lines 34-35. Can you also do a P for trend analysis (analysing categories of DR coded as an ordinal variable (coded 0 for no Dr, 1 for mild DR, 2 for moderate DR, and 3 for severe DR) with OCT and OCTA features. Please also add an explanation on this to the methods section.

13. Can you please elaborate on why individual quadrants were studied. Why would you expect associations to be different across individual quadrants? There is likely more

measurement error when investigating individual quadrants than when using the average value of all quadrants.

Discussion

14. page 13 paragraph 2, please add a comment on the sample sizes of previous studies and on whether these studies adjusted for potential confounders.

15. Page 14, paragraph 1. Do you know a biological explanation why inferior sector may be more susceptible to deterioration? If the physiology is unknown then please state this in this paragraph

16 Page 14, ines30-32; please replace controversially with ' in contrast'

17. Page 15, First limitation (lines 17-19) please add that temporality could not be accounted for.

Other comments that should be added to the limitations section is that no comments can be made on other ethnicities than the ethnicity in this study. Another limitation is that certain more sick individuals were excluded from the study population, hence this study may have underestimated associations under study.

Also, please elaborate on why it would be of use to investigate more peripherally located retinal layers (do you expect the periphery to more susceptible to ischemia? And if so, then why?)

18. Table 1 and 2: please add the N of individuals with different subtypes of DR to the table. This will make the tables more easy to interpret for readers.

Reviewer	**2**
Name	**El matri, Khaled**
Affiliation	**Institut Hedi Rais d'Ophtalmologie, Department B**
Date	**19-Feb-2024**
COI	**No interests**

I would like to congratulate the authors for this interesting well presented study.

I have few remarks prior to publication :

1- In methods-settings, Line 17: there is a typo-error with an extra "in". Please delete it.

2- Introduction, line 23: "microvascular lesions are present"

3- Did you exclude proliferative DR patients from the study? If yes, it should be mentioned in methods. Otherwise, how could you explain its absence in such a large cohort of diabetic patients.

4- In results, page 11, line 21: Authors stated: “In addition, VD in the peripapillary ring and the average peripapillary area decreased as the clinical manifestations of the DR worsened ». Please precise if this decrease was significant or not, between DR subgroups.

5- In results, page 11, lines 23-30: How could authors explain that VLD and VDI were significantly higher in RD group, while it should be the contrary.

6- In discussion, page 12, line 46: RPC has not been defined in the text.

7- Page 14, line 29: VAD has not been defined in the text.

8- The whole discussion should be revised.

9- Do authors suggest that RNFL analysis should be treated with caution in glaucoma suspicion, in diabetic patients with DR, since some alterations could be related to the DR itself.?

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1
Dr. Frank van der Heide , Maastricht University
Comments to the Author:
This population-based study among individuals with type 2 diabetes evaluated the association of stage of DR with OCTA measures (vascular density, vascular length density, vessel diameter index) and OCT measures (pRNFL), using data on 1,325 individuals with diabetes (n=210 with diabetes retinopathy and n=1,115 without diabetic retinopathy).
A major strength of the study is the large sample size.
A limitation of the study is that the authors did not evaluate interaction by age or sex. Further, there are several instances in which English language could be improved (although the English language is not bad in general).

Abstract
1. Methods section: Please add which confounders were used to adjust for in multivariable analyses.

[Response] Thank you very much for your valuable suggestion. We have incorporated the confounders that were used for adjustment in the multivariable analyses into the methods section of the main text. To review the details, please refer to page10, line 9-18.

2. Page 4 Line 57-59: The authors state that monitoring changes might be promising. This should be changed as no changes over time were studied in this study. Please use the word differences instead.

[Response] Thank you for bringing this to our attention. We have made the necessary revision and replaced the word “changes” with “differences” in the conclusion section of the abstract. You can find the updated wording in the abstract and the entire text. We appreciate your meticulousness and attention to detail.

3. Page 5 Strengths (line 27,28): community based design is not a limitation, please remove this comment here.

[Response] Thank you for your valuable suggestion. We have revised the manuscript as per your guidance and removed the term "community-based". We appreciate your guidance in refining the manuscript for improved accuracy and clarity.

Introduction

4. pg 6-7 Moreover, the peripapillary OCTA RNFL association during DR development and progression has not been fully addressed.Can you specify the literature in a more detailed way? What has exactly been looked at and what has not?

[Response] Thank you for your valuable suggestion. We have included specific references to the literature on the peripapillary OCTA-RNFL association in DR patients, as per your suggestion, with the aim to provide a clearer delineation of literature. Updated information can be referred on page 6 line 8-16. Now it reads:

To date, although researches have revealed that peripapillary microvascular parameters were correlated with the RNFL thickness in patients with DM,[1] the results of studies on the direct correlation between peripapillary vascular markers and RNFL thinning in patients with DR have been limited and inconclusive.[2,3]

Reference:

1. Lee MW, Lee WH, Ryu CK, Lee YM, Lee YH, Kim JY. Peripapillary Retinal Nerve Fiber Layer and Microvasculature in Prolonged Type 2 Diabetes Patients Without Clinical Diabetic Retinopathy. Invest Ophthalmol Vis Sci. 2021;62(2):9.
2. Shin YI, Nam KY, Lee SE, et al. Peripapillary microvasculature in patients with diabetes mellitus: An optical coherence tomography angiography study. Sci Rep. 2019;9(1):15814.
3. Vujosevic S, Muraca A, Gatti V, et al. Peripapillary Microvascular and Neural Changes in Diabetes Mellitus: An OCT-Angiography Study. Invest Ophthalmol Vis Sci. 2018;59(12):5074-5081.

5. pg 7 lines 15-16 'changes' – cross-sectional data were used so 'changes' were not measured, only 'differences', please rephrase.

[Response] Thank you for bringing this to our attention. We have made the requested change and replaced the word "changes" with "differences".

Methods

6. Page 8 lines 40-41, please explain abbreviations BCVA, SE

[Response] Thank you for the suggestions. The full words "best-corrected visual acuity (BCVA)" and "spherical equivalent (SE)" have been included where these abbreviations were first mentioned in the method section on Page 7, line 4-6. Please review the updated manuscript.

7. Page 9. Please explain in the ‘statistical analysis’ section which confounders were included in the models. Please also add some additional analyses for the supplemental material in which you evaluate the impact of some potential confounders other than age, sex on the associations (e.g. evaluating a model that contains duration of diabetes, HbA1C, body mass index, systolic blood pressure, and total cholesterol) and a model that contains axial length, intraocular pressure and OCTA signal strength intensity). The eye variables may not be potential confounders as although these variables are associated with OCT/OCTa features, they may not be associated with diabetes. Adjust for these variables may thus be overadjustment.

[Response] Thank you very much for your valuable suggestion. Firstly, I would like to state that the outcome variables (dependent variable) of the present study were various peripapillary OCTA parameters and RNFL thickness. Thus, in the multivariable linear regression model, we made adjustments for common confounding variables, which were potentially associated with RNFL/OCTA parameters in our Guangzhou Eye Study and other studies.
We made additional statistical analyses to assess the impact of the above mentioned potential confounders on RNFL/OCTA parameters with univariable linear regression. The results showed that beside total cholesterol and intraocular pressure, other factors were statistically associated with RNFL/OCTA parameters. Because there are many RNFL/OCTA variables, we presented the linear regression outcomes of PVD, PLD and RNFL in the average peripapillary area, please referred to table1 below for details. Although total cholesterol and intraocular pressure were not associated with RNFL/OCTA parameters in the present study, many previous studies found that total cholesterol and intraocular pressure were significantly associated with RNFL/OCTA parameters.[1-13] Therefore, we believed that it was necessary to include total cholesterol and intraocular pressure into the multivariable linear regression model to further exclude confounding effects and provide robust estimates of the associations. We also tried to remove total cholesterol and intraocular pressure from the multivariable linear regression, and the results were the same, please referred to table2 below for details.
Axial length, intraocular pressure and OCTA signal strength intensity are associated with RNFL/OCTA features. Thus, it should be adjusted in the multivariable linear regression model. We believed that adjustment for these variables may be not overadjustment. We have added the information on confounders that were utilized to adjust for in the multivariable analyses in the methods section. Please refer to page 10, line 9-18 to review the specific details.

Univariable and multivariable linear regression analyses were used to evaluate the associations of the peripapillary OCTA parameters (VD, VLD, and VDI) with the various stages of DR and RNFL thickness after adjustments for age, sex, duration of diabetes, HbA1C level, body mass index, systolic blood pressure, total cholesterol level, axial length, intraocular pressure and OCTA signal strength intensity. These confounding factors were chosen on the basis of the Guangzhou Diabetic Eye Study and other studies.[1,2,5,10,11,13]

Reference
1. Ding Q, Wu H, Wang W, et al. Association of Body Mass Index and Waist-to-Hip Ratio With Retinal Microvasculature in Healthy Chinese Adults: An Optical

Coherence Tomography Angiography Study. Am J Ophthalmol. Feb 2023;246:96-106.
2. Sampson DM, Gong P, An D, et al. Axial Length Variation Impacts on Superficial Retinal Vessel Density and Foveal Avascular Zone Area Measurements Using Optical Coherence Tomography Angiography. Invest Ophthalmol Vis Sci. Jun 1 2017;58(7):3065-3072.
3.Eid P, Arnould L, Gabrielle PH, Aho LS, Farnier M, Creuzot-Garcher C, Cottin Y. Retinal Microvascular Changes in Familial Hypercholesterolemia: Analysis with Swept-Source Optical Coherence Tomography Angiography. J Pers Med. 2022 May 26;12(6):871.
4.Shi R, Lu Y, Liu D, Guo Z. Association of serum apolipoprotein B with retinal neurovascular structural alterations in patients with type 2 diabetes: an optical coherence tomography angiography study. Acta Diabetol. 2021 Dec;58(12):1673-1681.
5.Lee DH, Yi HC, Bae SH, Cho JH, Choi SW, Kim H. Risk factors for retinal microvascular impairment in type 2 diabetic patients without diabetic retinopathy. PLoS One. 2018 Aug 9;13(8):e0202103.
6.Chuang LH, Li JH, Huang PW, Chen HSL, Liu CF, Yang JW, Lai CC. Association of Intraocular Pressure and Optical Coherence Tomography Angiography Parameters in Early Glaucoma Treatment. Diagnostics (Basel). 2022 Sep 8;12(9):2174.
7.Wu Y, Yang Q, Ding L, Tu Y, Deng X, Yang Y, Shen M, Lu Q, Lu F, Chen Q. Peripapillary structural and microvascular alterations in early dysthyroid optic neuropathy. Eye Vis (Lond). 2022 Aug 9;9(1):30.
8.Liu C, Umapathi RM, Atalay E, Schmetterer L, Husain R, Boey PY, Aung T, Nongpiur ME. The Effect of Medical Lowering of Intraocular Pressure on Peripapillary and Macular Blood Flow as Measured by Optical Coherence Tomography Angiography in Treatment-naive Eyes. J Glaucoma. 2021 Jun 1;30(6):465-472.
9.Cheng W, Song Y, Lin F, Jin L, Wang Z, Jonas JB, Wang W, Zhang X. Choriocapillaris Flow Deficits in Normal Chinese Imaged by Swept-Source Optical Coherence Tomographic Angiography. Am J Ophthalmol. 2022 Mar;235:143-153.
10.Song Y, Cheng W, Li F, Lin F, Wang P, Gao X, Peng Y, Liu Y, Zhang H, Chen S, Fan Y, Zhang R, Wang W, Zhang X. Ocular Factors of Fractal Dimension and Blood Vessel Tortuosity Derived From OCTA in a Healthy Chinese Population. Transl Vis Sci Technol. 2022 May 2;11(5):1.
11.Lal B, Alonso-Caneiro D, Read SA, Tran B, Van Bui C, Tang D, Fiedler JT, Ho S, Carkeet A. Changes in Retinal Optical Coherence Tomography Angiography Indexes Over 24 Hours. Invest Ophthalmol Vis Sci. 2022 Mar 2;63(3):25.
12.Lamparter J, Schmidtmann I, Schuster AK, Siouli A, Wasielica-Poslednik J, Mirshahi A, Höhn R, Unterrainer J, Wild PS, Binder H, Lackner K, Beutel ME, Münzel T, Pfeiffer N, Hoffmann EM. Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness. PLoS One. 2018 May 22;13(5):e0197682.
13.Wu J, Du Y, Lin C, Zhu Y, Chen W, Pan Q, Zhuo Y, Wang N. Retinal nerve fibre layer thickness measured with SD-OCT in a population-based study: the Handan Eye Study. Br J Ophthalmol. 2023 Aug;107(8):1156-1164.

Table 1. Univariable linear regression of peripapillary retinal microcirculation and RNFL with basic characteristics

Characteristics	PVD in the average peripapillary area		PVLD in the average peripapillary area		RNFL in the average peripapillary area	
	β (95% CI)	P value	β(95% CI)	P value	β(95% CI)	P value
Age,year	-0.07 (-0.09, -0.05)	**<0.001**	-0.03 (-0.04, -0.01)	**<0.001**	-0.28 (-0.37, -0.20)	**<0.001**
Sex	-0.68(-0.97, -0.39)	**<0.001**	-0.36(-0.55, -0.16)	**<0.001**	-1.19(-2.50, 0.13)	0.076
Duration, year	-0.04(-0.06, -0.02)	**<0.001**	-0.02(-0.04, -0.01)	**0.003**	-0.14(-0.23, -0.05)	**0.004**
HbA1c, %	-0.20(-0.30, -0.10)	**<0.001**	-0.08(-0.14, -0.01)	**0.029**	-0.52(-0.95, -0.08)	**0.021**
BMI, kg/m^2	0.06(0.01, 0.11)	**0.015**	0.04(0.01, 0.07)	**0.028**	0.13(-0.16,0.42)	0.378
Systolic blood pressure, mmHg	-0.01(-0.02, -0.01)	**0.001**	0.01(0.00, 0.02)	**0.046**	-0.07(-0.10, -0.03)	**<0.001**
Total cholesterol, mmol/L	0.06(-0.08, 0.20)	0.376	0.04(-0.05, 0.14)	0.401	-0.06(-0.68, 0.55)	0.840
AL, mm	-0.35(-0.52, -0.19)	**<0.001**	-0.15(-0.27, -0.03)	**0.011**	-1.85(-2.60, -1.11)	**<0.001**
IOP, mmHg	0.03(-0.02, 0.08)	0.314	0.01(-0.02, 0.05)	0.507	-0.07(-0.30, 0.16)	0.565
OCTA signal strength intensity	0.06(0.05, 0.08)	**<0.001**	0.04(0.03, 0.05)	**<0.001**	0.12(0.01,0.24)	**0.047**

Bold indicates statistical significance.

Abbreviations: *VD= vessel density; VLD=vessel length density; RNFL= retinal nerve fiber layer; BMI=body mass index; AL=axial length;IOP=intraocular pressure; OCTA= optical coherence tomography angiography.*

Table 2. Multivariable linear regression of peripapillary retinal microcirculation and RNFL with various stages of DR

Parameters	Non-DR vs. Mild DR		Non-DR vs. Moderate DR		Non-DR vs. Severe DR	
	β (95% CI)	P value	β(95% CI)	P value	β(95% CI)	P value
VD(wi)	0.60(-0.14, 1.35)	0.111	-0.49(-0.98, 0.001)	0.050	-0.10(-0.95, 0.76)	0.826
VLD(wi)	0.55(0.03, 1.06)	**0.037**	-0.40(-0.74, -0.06)	**0.020**	-0.06(-0.65, 0.53)	0.836
VD(cir)	-0.25(-1.14, 0.62)	0.568	-0.72(-1.30, -0.14)	**0.015**	-1.80(-2.81, -0.79)	**0.001**

VLD(cir)	-0.06(-0.66, 0.54)	0.841	-0.42(-0.82, -0.03)	**0.036**	-1.22(-1.91, -0.53)	**0.001**
VDI(cir)	0.002(0.00003, 0.003)	**0.045**	0.001(-0.0001, 0.002)	0.084	0.001(-0.001, 0.002)	0.416
VD(average)	0.07(-0.68, 0.82)	0.854	-0.75(-1.24, -0.25)	**0.003**	-1.78(-2.64, -0.92)	**<0.001**
VLD(average)	0.19(-0.34, 0.72)	0.487	-0.46(-0.81, -0.11)	**0.011**	-1.26(-1.87, -0.65)	**<0.001**
VD(s)	-0.25(-1.69, 1.19)	0.733	-0.44(-1.39, 0.51)	0.360	-2.71(-4.37, -1.05)	**0.001**
VD(n)	-0.25(-1.79, 1.30)	0.754	-1.08(-2.10, -0.07)	**0.037**	-2.32(-4.10, -0.54)	**0.011**
VD(i)	0.38(-1.18, 1.94)	0.634	-1.10(-2.13, -0.07)	**0.036**	-1.96(-3.76, -0.17)	**0.032**
VD(t)	-0.88(-2.48, 0.71)	0.277	-0.25(-1.30, 0.79)	0.636	-0.20(-2.04, 1.63)	0.827
VLD(s)	-0.01(-0.92, 0.90)	0.983	-0.20(-0.80, 0.40)	0.508	-1.62(-2.67, -0.57)	**0.002**
VLD(n)	-0.08(-1.05, 0.90)	0.876	-0.69(-1.34, -0.05)	**0.034**	-1.48(-2.61, -0.36)	**0.010**
VLD(i)	0.44(-0.56, 1.44)	0.390	-0.66(-1.32, 0.01)	0.050	-1.34(-2.49, -0.19)	**0.022**
VLD(t)	-0.57(-1.58, 0.44)	0.269	-0.13(-0.80, 0.53)	0.695	-0.44(-1.60, 0.73)	0.462
RNFL(average)	0.45(-2.96, 3.86)	0.796	-1.97(-4.23, 0.30)	0.089	-2.14(-6.32, 2.04)	0.315
RNFL(s)	-0.99(-6.71, 4.73)	0.734	-3.22(-7.02, 0.59)	0.097	-5.36(-12.38, 1.66)	0.134
RNFL(n)	-0.57(-5.88, 4.74)	0.834	-0.37(-3.91, 3.16)	0.837	8.95(2.43, 15.48)	**0.007**
RNFL(i)	3.16(-3.46, 9.78)	0.349	-4.42(-8.82, -0.01)	**0.049**	-10.27(-18.39, -2.14)	**0.013**
RNFL(t)	0.13(-5.33, 5.60)	0.962	0.11(-3.53, 3.75)	0.953	-2.26(-8.97, 4.45)	0.508

Adjusted for *age, sex, duration of diabetes, HbA1C, body mass index, systolic blood pressure, axial length and OCTA signal strength intensity.*
Bold indicates statistical significance.
Abbreviations: *RNFL= retinal nerve fiber layer; DR=diabetic retinopathy; VD= vessel density; VLD=vessel length density; VDI= vessel density index; s=superior; i=inferior; n=nasal; t=temporal; wi=the whole image; circ=peripapillary ring; average=the entire peripapillary area;OCTA= optical coherence tomography angiography*

8. Page 9 Please test whether associations differ between men and women by testing for interaction (include an interaction of e.g. sex*VD in the model and evaluate whether the P-value of this interaction term is <0.05. And in the case of interaction show stratified analyses)

[Response] Thanks very much for pointing out that interaction of sex may affect the accuracy of the result. The outcome variables (dependent variable) of the present study were peripapillary OCTA parameters and RNFL thickness. Thus, we made the statistical analysis of the interaction of sex and severity of DR on all OCTA parameters and RNFL, and the results indicated no interaction (all P>0.05).

Results

9. Page 9 Please add a flowchart (can be put in main manuscript or supplement).

[Response] Thanks very much for pointing out our insufficient. We have added a flowchart and updated it as Figure1in the manuscript, as per your recommendation. We appreciate your guidance to further improve the manuscript.

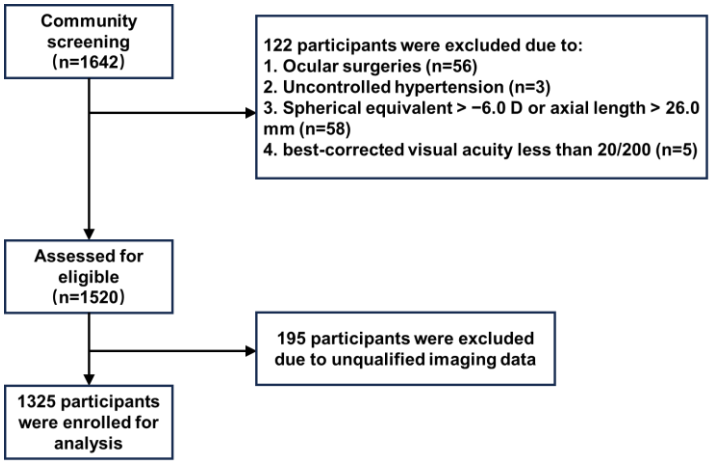

Figure1. Flowchart of the included participants

10. Please clarify de abbreviation LT on page 11 lines7-9.

[Response] The full words "Lens thickness" have been included where the abbreviation first appeared in the Method section on page8, line6.

11. Page 11 paragraph starting on lines13-15. Please specify that this paragraph refers to univariable analyses

[Response] Thanks very much for pointing out this question. The paragraph you mentioned used Student's t-test analyses to compare the differences of the peripapillary RNFL / OCTA parameters between individuals with DR and those without DR, rather than conducting univariable analyses. We have added a footnote in table1 to clarify it.

12. Page 11 paragraph starting on lines 34-35. Can you also do a P for trend analysis (analysing categories of DR coded as an ordinal variable (coded 0 for no Dr, 1 for mild DR, 2 for moderate DR, and 3 for severe DR) with OCT and OCTA features. Please also add an explanation on this to the methods section.

[Response] Thanks a lot for your thoughtful suggestion. A P value for trend has been calculated and added to Table 2 according to your suggestion. We have updated the corresponding information in the methods and result section.

Methods section, page10, line18-19

The linear trend was examined using the medians of the OCT/OCTA parameters.

Results section, page11, line13-31 and line49-52

In the multivariable logistic regression model, the participants with moderate and severe DR showed progressively decreased VDs in the peripapillary ring (β = -0.72 for moderate, P = 0.015; β = -1.79 for severe, P = 0.001;P for trend<0.001), average peripapillary area (β = -0.74 for moderate, P = 0.003; β = -1.78 for severe, P < 0.001;P for trend<0.001), nasal quadrant (β = -1.09 for moderate, P = 0.037; β = -2.39 for severe, P = 0.009;P for trend<0.001), and inferior quadrant of the ONH (β = -1.14 for moderate, P = 0.03; β = -2.00 for severe, P = 0.03;P for trend<0.001) compared with those without DR.

In the multivariable logistic regression model, the progressively thinner peripapillary RNFL in the inferior quadrant was significantly associated with moderate (β = -4.56, P = 0.043) and severe DR (β = -10.12, P = 0.015) (P for trend=0.03

Table 2. Multivariable linear regression of peripapillary retinal microcirculation and RNFL with various stages of DR

Parameters	Non-DR vs. Mild DR		Non-DR vs. Moderate DR		Non-DR vs. Severe DR		P value for trends
	β (95% CI)	P value	β(95% CI)	P value	β(95% CI)	P value	
VD(wi)	0.61(-0.13, 1.36)	0.107	-0.49(-0.98, -0.001)	**0.049**	-0.07(-0.93, 0.79)	0.873	0.091
VLD(wi)	0.55(0.03, 1.06)	**0.037**	-0.40(-0.74, 0.07)	**0.019**	-0.06(-0.66, 0.54)	0.839	0.118
VD(cir)	-0.25(-1.14, 0.63)	0.571	-0.72(-1.30, -0.14)	**0.015**	-1.79(-2.81, -0.77)	**0.001**	**<0.001**
VLD(cir)	-0.06(-0.67, 0.54)	0.835	-0.43(-0.82, 0.03)	**0.035**	-1.23(-1.92, -0.53)	**0.001**	**<0.001**
VDI(cir)	0.002(0.00004, 0.003)	**0.044**	0.001(-0.0001, 0.002)	0.082	0.001(-0.001, 0.002)	0.417	0.149
VD(average)	0.07(-0.68, 0.82)	0.855	-0.74(-1.24, -0.25)	**0.003**	-1.78(-2.65, -0.91)	**<0.001**	**<0.001**
VLD(average)	0.19(-0.35, 0.72)	0.494	-0.45(-0.80, -0.10)	**0.011**	-1.27(-1.89, -0.65)	**<0.001**	**<0.001**
VD(s)	-0.21(-1.66, 1.23)	0.77	-0.41(-1.36, 0.54)	0.399	-2.58(-4.25, -0.91)	**0.003**	**0.001**
VD(n)	-0.26(-1.81, 1.29)	0.741	-1.09(-2.11, -0.07)	**0.037**	-2.39(-4.18, -0.59)	**0.009**	**<0.001**
VD(i)	0.36(-1.20, 1.92)	0.652	-1.14(-2.16, -0.11)	**0.03**	-2.00(-3.81, -0.20)	**0.03**	**<0.001**
VD(t)	-0.88(-2.47, 0.72)	0.28	-0.25(-1.30, 0.80)	0.636	-0.18(-2.03, 1.66)	0.845	**0.011**
VLD(s)	0.01(-0.90, 0.92)	0.986	-0.18(-0.79, 0.42)	0.546	-1.56(-2.61, -0.50)	**0.004**	**0.004**
VLD(n)	-0.09(-1.07, 0.89)	0.862	-0.70(-1.34, -0.05)	**0.034**	-1.53(-2.66, -0.39)	**0.008**	**<0.001**
VLD(i)	0.42(-0.58, 1.42)	0.408	-0.68(-1.34, -0.02)	**0.043**	-1.38(-2.54, -0.23)	**0.019**	**<0.001**
VLD(t)	-0.57(-1.58, 0.44)	0.268	-0.14(-0.80, 0.53)	0.686	-0.44(-1.62, 0.73)	0.46	**0.021**
RNFL(average)	0.40(-3.01, 3.80)	0.82	-1.99(-4.26, 0.27)	0.085	-2.41(-6.62, 1.79)	0.261	**0.029**

RNFL(s)	*-1.13(-6.84, 4.58)*	*0.698*	*-3.14(-6.95, 0.66)*	*0.105*	*-6.19(-13.24, 0.87)*	*0.086*	*0.150*
RNFL(n)	*-0.65(-5.97, 4.67)*	*0.81*	*-0.41(-3.95, 3.13)*	*0.821*	*-8.61(2.04, 15.18)*	***0.01***	*0.862*
RNFL(i)	*3.17(-3.45, 9.79)*	*0.347*	*-4.56(-8.97, -0.15)*	***0.043***	*-10.12(-18.29, -1.95)*	***0.015***	***0.03***
RNFL(t)	*0.13(-5.34, 5.60)*	*0.963*	*0.10(-3.54, 3.75)*	*0.956*	*-2.34(-9.10, 4.41)*	*0.497*	*0.612*

Adjusted for *age, sex, duration of diabetes, HbA1C, body mass index, systolic blood pressure, total cholesterol, axial length, intraocular pressure and OCTA signal strength intensity.*

Bold indicates statistical significance.

Abbreviations: *RNFL= retinal nerve fiber layer; DR=diabetic retinopathy; VD= vessel density; VLD=vessel length density; VDI= vessel density index; s=superior; i=inferior; n=nasal; t=temporal; wi=the whole image; circ=peripapillary ring; average=the entire peripapillary area;OCTA= optical coherence tomography angiography*

13. Can you please elaborate on why individual quadrants were studied. Why would you expect associations to be different across individual quadrants? There is likely more measurement error when investigating individual quadrants than when using the average value of all quadrants.

[Response] Thanks very much for pointing out this important question. Quadrants differences of retinal structure and function exist in ocular disease. For example, different stage and severity of glaucoma can have an unbalanced impact on the RNFL in various subregions. Typically, glaucoma leads to progressive thinning of the RNFL in the superior and inferior quadrants at earlier stage, and as the disease advances, it may gradually involve other quadrants as well.[1,2] Studies have reported that glaucoma patients exhibit lower peripapillary blood flow density, with more pronounced reductions observed in the superior and inferior regions.[3]
As to DM, a study reported a significant difference in the RNFL of the inferior quadrants among DR, non-DR, and normal control participants.[4] Lee et al. reported that the DM group had a thinner inferior RNFL compared to the healthy group, and this thinning was more severe in patients with DM for longer than 10 years without DR.[5] Thus, we aim to investigate whether there are quadrant-specific differences in RNFL-OCTA associations at in different stages of DR.
Furthermore, OCT/OCTA parameters of subregions are now widely used in predicting ocular and systemic diseases,[6-9] the measurement error is within the permissible limits, and the accuracy and reliability of which have been validated and proven trustworthy.

Reference:
1.Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet. 2004 May 22;363(9422):1711-20. doi: 10.1016/S0140-6736(04)16257-0.
2.Maupin E, Baudin F, Arnould L, Seydou A, Binquet C, Bron AM, Creuzot-Garcher CP. Accuracy of the ISNT rule and its variants for differentiating glaucomatous from

normal eyes in a population-based study. Br J Ophthalmol. 2020 Oct;104(10):1412-1417.
3. Yospon T, Rojananuangnit K. Optical Coherence Tomography Angiography (OCTA) Differences in Vessel Perfusion Density and Flux Index of the Optic Nerve and Peri-Papillary Area in Healthy, Glaucoma Suspect and Glaucomatous Eyes. Clin Ophthalmol. 2023 Oct 12;17:3011-3021.
4.Vujosevic S, Muraca A, Gatti V, et al. Peripapillary Microvascular and Neural Changes in Diabetes Mellitus: An OCT-Angiography Study. Invest Ophthalmol Vis Sci. 2018;59(12):5074-5081.
5. Lee MW, Lee WH, Ryu CK, Lee YM, Lee YH, Kim JY. Peripapillary Retinal Nerve Fiber Layer and Microvasculature in Prolonged Type 2 Diabetes Patients Without Clinical Diabetic Retinopathy. Invest Ophthalmol Vis Sci. 2021;62(2):9.
6. Sun Y, Zhang L, Ye H, Leng L, Chen Y, Su Y, Ren P, Lu H, Peng G. Potential ocular indicators to distinguish posterior cortical atrophy and typical Alzheimer's disease: a cross-section study using optical coherence tomography angiography. Alzheimers Res Ther. 2024 Mar 25;16(1):64.
7. Nam J, Nivison-Smith L, Trinh M. Spatial Analysis Reveals Vascular Changes in Retinal and Choroidal Vessel Perfusion in Intermediate AMD With Reticular Pseudodrusen. Invest Ophthalmol Vis Sci. 2024 Feb 1;65(2):33.
8. Meng L, Chen L, Zhang C, Chen H, Yang J, Wang Y, Zhang W, Cheng S, Zhao Q, Zhao X, Chen Y. Quantitative assessment of retinal vasculature changes in systemic lupus erythematosus using wide-field OCTA and the correlation with disease activity. Front Immunol. 2024 Jan 29;15:1340224.
9. Yuan M, Wang W, Kang S, et al. Peripapillary Microvasculature Predicts the Incidence and Development of Diabetic Retinopathy: An SS-OCTA Study. Am J Ophthalmol. Nov 2022;243:19-27.

Discussion
14. page 13 paragraph 2, please add a comment on the sample sizes of previous studies and on whether these studies adjusted for potential confounders.

[Response] Thank you for your valuable suggestion. We have added a comment on the sample sizes of previous studies and whether these studies adjusted for potential confounders in the manuscript according to your suggestion. Please refer to page13 line 11-18.

However, these studies had non-Chinese participants and small sample sizes. In addition, Vujosevic did not adjust for any potential confounders, while Shin made adjustments only for the macular ganglion cell-inner plexiform layer and RNFL.

15. Page 14, paragraph 1. Do you know a biological explanation why inferior sector may be more susceptible to deterioration? If the physiology is unknown then please state this in this paragraph.

[Response] Previous studies have indicated that the inferior sector of ONH had a lower blood flow per unit nerve tissue volume compared to the superior sector, suggesting that the inferior peripapillary area is more susceptible to ischemic insults.[1,2] Consequently, the inferior sector may be more prone to deterioration. We have added this clarification on page14, line 23-30 to provide a better explanation of

our results. Now it reads:

The possible mechanism of the higher susceptibility in the inferior sector maybe that the inferior peripapillary area is more vulnerable to ischemic insults because the inferior sector of the ONH had lower blood flow than the superior sector.[1,2]

Reference
1. Harris A, Ishii Y, Chung HS, Jonescu-Cuypers CP, McCranor LJ, Kagemann L, Garzozi HJ. Blood flow per unit retinal nerve fibre tissue volume is lower in the human inferior retina. Br J Ophthalmol. 2003 Feb;87(2):184-8.
2.Tomita R, Iwase T, Ueno Y, Goto K, Yamamoto K, Ra E, Terasaki H. Differences in Blood Flow Between Superior and Inferior Retinal Hemispheres. Invest Ophthalmol Vis Sci. 2020 May 11;61(5):27.

16 Page 14, lines30-32; please replace controversially with ‘ in contrast’

[Response] Thank you for your kind suggestion. We have replaced "controversially" with "in contrast" in the manuscript. Please check it.

17. Page 15, First limitation (lines 17-19) please add that temporality could not be accounted for.
Other comments that should be added to the limitations section is that no comments can be made on other ethnicities than the ethnicity in this study. Another limitation is that certain more sick individuals were excluded from the study population, hence this study may have underestimated associations under study.
Also, please elaborate on why it would be of use to investigate more peripherally located retinal layers (do you expect the periphery to more susceptible to ischemia? And if so, then why?)

[Response] Thank you for your meaningful suggestion. The sentence “temporality could not be accounted for” has been included in the first limitation on page 15, line 32-37.
Additionally, comments on other ethnicities have been added in the second limitation on page 15, line 37-40.
Regarding the exclusion of more severely ill individuals from the study population, we agreed with you that this may have led to an underestimation of the associations under investigation. However, despite this limitation, the study’s conclusion remains a meaningful reference due to its large population size. We have included it as another limitation on page15, line 41-45.
As it is well-known, diabetic retinopathy can affect the entire retina. We recognize the importance of exploring retinal layers in a larger area to gain a better understanding of the mechanisms involved in the development and progression of DR. However, the OCTA scanning range of the current study was limited to 3- × 3 mm. The word “peripheral” here is inappropriate, and we have revised it to “peripapillary retinal layers with DR in a larger region”. These changes have been made in the manuscript on page 15, line 45-51. The revised limitation sector now reads:

First, as it is a cross-sectional study, temporality could not be accounted for, it was difficult to illustrate the causal relationship between peripapillary RNFL

thinning and decreased peripapillary perfusion. Another limitation is that it is a community-based study with participants from a single city. Therefore, generalizations to other areas and ethnicities are limited. Third, severely sick individuals were excluded from the study population. Thus, we believe that the results might have underestimated the association but still have an important reference value. Finally, we could not explore the association of the peripapillary retinal layers with DR in a larger region because only 3- x 3 mm scanned ONH images were included.

18. Table 1 and 2: please add the N of individuals with different subtypes of DR to the table. This will make the tables more easy to interpret for readers.

[Response] Thank you for your suggestion. We have added the number of individuals with different subtypes of DR into table1 and table2, as recommended. This addition will enhance the interpretability of the table for readers, providing value context regarding the distribution of DR subtypes within our study population. We appreciate your feedback and we have taken care to accurately reflect this information in the revised tables.

Reviewer: 2
Dr. Khaled El matri, Institut Hedi Rais d'Ophtalmologie
Comments to the Author:
I would like to congratulate the authors for this interesting well presented study.
I have few remarks prior to publication :

1- In methods-settings, Line 17: there is a typo-error with an extra “in”. Please delete it.

[Response] Thank you for pointing out the error. We have removed the extra “in” in the methods-settings section. Your attention to detail is much appreciated.

2- Introduction, line 23: “microvascular lesions are present”

[Response] Thank you for pointing out the error. We have changed the word “presented” to “present”.

3- Did you exclude proliferative DR patients from the study? If yes, it should be mentioned in methods. Otherwise, how could you explain its absence in such a large cohort of diabetic patients.

[Response] We did not exclude proliferative DR patients in the current study. Actually, severity of DR was categorized into no retinopathy, mild, moderate, and severe DR according to the American Academy of Ophthalmology (AAO) International Clinical Diabetic Retinopathy Disease Severity Scale.[1] According to AAO criteria, severe DR encompasses both severe nonproliferative DR and proliferative DR.

Reference

1. Wilkinson CP, Ferris FL, 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677-1682.

4- In results, page 11, line 21: Authors stated: “In addition, VD in the peripapillary ring and the average peripapillary area decreased as the clinical manifestations of the DR worsened ». Please precise if this decrease was significant or not, between DR subgroups.

[Response] Thank you for your attention to detail. We have added the P value to indicate the statistical significance of the VD decrease between DR subgroups. Now it reads:
In addition, the VDs in the peripapillary ring (P <0.001) and average peripapillary area (P <0.001) decreased as the clinical manifestations of DR worsened (Figure 2).

5- In results, page 11, lines 23-30: How could authors explain that VLD and VDI were significantly higher in RD group, while it should be the contrary.

[Response] Thanks very much for your thoughtful comment. We apologize for the previous mistake in writing the results on VLD in DR group. Based on table1, we have corrected the statement to reflect that VLD in the peripapillary ring, average peripapillary area, and each subquadrant of the ONH were all significantly lower in the DR group compared to the non-DR group (all P < 0.05). This correction has been made on page11, line 4-8.

As to VDI, which represents the average vessel calibre of the blood vessels, it is known to be sensitive to vascular dilation in OCTA images as it provides the vessel size information regardless of the vessel length. Previous studies have shown that increased VDI was correlated with higher fasting glucose levels.[1,2] Our study revealed a slightly higher VDI in the DR group compared to the non-DR group. We hypothesized that this observation may be attributed, at least in part, to the compensatory vasodilation of perfused capillaries secondary to a hypoxic environment or increased local inflammatory molecules. Additionally, it is noteworthy that the blood vessel caliber measurements may not be entirely accurate with OCTA imaging.[3] We have incorporated this discussion on page 13, line 41-59. Now it reads:

However, peripapillary VDI, an index of vascular caliber, was slightly higher in the DR group in the current study, which is consistent with the results of previous studies that indicated that increased VDI correlated with higher fasting glucose levels.[1,2] We hypothesized that a hypoxic glose environment or increased local inflammatory molecules induces a compensatory vasodilation of the capillaries. By contrast, an observational study with small samples found that VDI is reduced in DR, without adjustments for any confounding factors.[3] Moreover, blood vessel caliber measurements on OCTA imaging may not be accurate.[4]

Reference:
1. Alam, M., Thapa, D., Lim, J.I., Cao, D., Yao, X., 2017. Quantitative characteristics of sickle cell retinopathy in optical coherence tomography angiography. Biomed. Opt Express 8, 1741–1753.
2. Tang FY, Ng DS, Lam A, Luk F, Wong R, Chan C, Mohamed S, Fong A, Lok J, Tso T, Lai F, Brelen M, Wong TY, Tham CC, Cheung CY. Determinants of Quantitative Optical Coherence Tomography Angiography Metrics in Patients with

Diabetes. Sci Rep. 2017 May 31;7(1):2575.
3. Ghasemi Falavarjani K, Al-Sheikh M, Darvizeh F, Sadun AA, Sadda SR. Retinal vessel calibre measurements by optical coherence tomography angiography. Br J Ophthalmol 2017;101:989-992
4.Frizziero L, Parrozzani R, Londei D, Pilotto E, Midena E. Quantification of vascular and neuronal changes in the peripapillary retinal area secondary to diabetic retinopathy. Br J Ophthalmol. Nov 2021;105(11):1577-1583.

6- In discussion, page 12, line 46: RPC has not been defined in the text.

[Response] Thank you for bringing this to our attention. We have defined RPC (Radial Peripapillary Capillary) upon its first mention in the discussion section on page 12, line 28.

7- Page 14, line 29: VAD has not been defined in the text.

[Response] Thank you for bringing this to our attention. We have revised the abbreviation "VAD" to "vessel area density" in the text as there is no subsequent mention of the abbreviation "VAD" in the manuscript.

8- The whole discussion should be revised.
[Response] We have added some discussion and revised the language errors and spelling mistakes of the discussion section thoroughly. We appreciate your thorough review and attention to detail.

9- Do authors suggest that RNFL analysis should be treated with caution in glaucoma suspicion, in diabetic patients with DR, since some alterations could be related to the DR itself?

[Response] As we all know, glaucoma typically results in progressive thinning of RNFL in the superior and inferior quadrants at the beginning stage. In addition, the current study has indicated that the RNFL thinning in the inferior quadrant was associated with moderate and severe DR. Thus, it's important to acknowledge that alterations in RNFL thickness may not solely reflect glaucomatous damage but could also be influenced by the underlying diabetic retinopathy in diabetic patients. For example, RNFL thinning may occur due to vascular ischemia, diabetic optic neurodegeneration, or other pathological processes of DR. Therefore, caution should be exercised when interpreting RNFL analysis in cases of suspected glaucoma among diabetic patients with DR.

VERSION 2 - REVIEW

Reviewer	**3**
Name	**Scandrett, Katie**
Affiliation	**University of Birmingham**
Date	**13-Nov-2024**

COI **N/A**

Thank you for the opportunity to review this interesting submission. Whilst the majority of the manuscript is well-written, the statistical methods section lacks detail and as a result, the results section is unclear. In particular, I question whether logistic regression has been used to generate the results presented in Table 2 instead of linear regression. Please see my comments below.

Main comments

Please include confidence intervals for the effect measures in the abstract and throughout the paper rather than just reporting p-values.

The following sentence is unclear: 'The data from the eyes with more severe DR were analyzed'. Are the authors referring two how one eye was included per patient, and the more severe eye was chosen? If both eyes were included per patient, a method to account for this correlation between observations from the same individuals in the linear regression model would need to be used.

Why were separate models created for each disease (diabetic retinopathy) category? It is not mentioned in the methods section that the data was split by disease stage. Why was this method chosen over including a covariate for diabetic retinopathy stage in the model?

As mentioned in my previous comment, multiple regression models have been fitted to different subsets of the data. Additionally, many statistical tests for trend have been conducted. This will increase the probability of a type I error occurring. If no adjustment for multiple testing is made, this should at least be mentioned as a limitation of the study.

No sample size calculation is mentioned.

Please include in the methods section which parameters were included in the regression models.

Which median values were compared using the test for trend? Are these the median values for each parameter in each subgroup of patients presented in Table 2? This is unclear from the methods section.

There is no mention of multicollinearity throughout and how this many affect the results and the conclusions that can be drawn from the results. For example, are the whole image, peripapillary ring, and the entire peripapillary area not likely to be correlated? Or have separate regression models been generated for each parameter, rather than including all parameters in one model? This is unclear.

Two sets of multivariable regression models have been presented in Tables 2 and 3. Are the models in Table 3 fitted to the whole dataset? This is unclear and is not described in the

methods section. Why is RNFL included in Table 2 as a 'parameter'? The methods section suggests that this is the outcome of the model.

Including 'Non-DR vs. Mild DR' in Table 2 is confusing. The reader may interpret this as a logistic regression model comparing the peripapillary retinal microcirculation in patients with and without diabetic retinopathy. However as mentioned above, since RNFL is included as covariate in the model I question whether results in Table 2 are from a logistic regression model, using diabetic retinopathy status as the outcome. The results described on page 11 also suggest that a logistic regression model may have been used.

Minor comments

It is unclear from the abstract that RNFL thickness is the outcome in the regression models. Or as mentioned in my previous comments, perhaps the outcome for some of the models was diabetic retinopathy stage?

How were patients sampled; were they sampled consecutively or randomly?

What is 'systemic' information?

In Figure 1 it is apparent that 195 participants were excluded due to unqualified imaging data. Did the authors investigate whether the characteristics of these patients differed significantly to the rest of the cohort?

Perhaps the table of patient characteristics could be included in the main manuscript rather than the supplementary material.

The Kolmogorov–Smirnov test can be too conservative with low power to detect skewed distributions. Instead, the distribution of a variable can be assessed from inspection of a histogram. Also, the normality should be assessed within each group (i.e. those with/without diabetic retinopathy). If the normality assumption is not met, a non-parametric test can be used.

Which RNFL covariate was used as the outcome for the regression model presented in Table 3 (average, S, N, I, T)?

The study is described as a 'large cohort study' in the discussion. This is misleading since the study was cross-sectional.

How accurate is the OCT imaging and image analysis software? Perhaps this should be mentioned in the discussion, potentially as a limitation.

VERSION 2 - AUTHOR RESPONSE

Reviewer: 3
Miss Katie Scandrett, University of Birmingham
Comments to the Author:

Thank you for the opportunity to review this interesting submission. Whilst the majority of the manuscript is well-written, the statistical methods section lacks detail and as a result, the results section is unclear. In particular, I question whether logistic regression has been used to generate the results presented in Table 2 instead of linear regression. Please see my comments below.

Main comments

Please include confidence intervals for the effect measures in the abstract and throughout the paper rather than just reporting p-values.
[Response] We appreciate this suggestion. We have updated the manuscript to include confidence intervals for the effect measures in both the abstract and throughout the paper. We believe this enhances the clarity and precision of the results. Please take a moment to review the changes.

The following sentence is unclear: 'The data from the eyes with more severe DR were analyzed'. Are the authors referring two how one eye was included per patient, and the more severe eye was chosen? If both eyes were included per patient, a method to account for this correlation between observations from the same individuals in the linear regression model would need to be used.
[Response] We appreciate the opportunity to clarify this statement. Your interpretation was correct; only one eye was included per patient in the current study. We have revised the sentence for clarity on page 9 line 47-51. Now it reads:
For those with bilateral DR, the eyes with more severe DR were analyzed. In cases where both eyes were at the same stage or only the right eye was available, we used data from the right eye.

Why were separate models created for each disease (diabetic retinopathy) category? It is not mentioned in the methods section that the data was split by disease stage. Why was this method chosen over including a covariate for diabetic retinopathy stage in the model?
[Response] Thank you for raising this question. We apologize for any misunderstanding regarding the tables. We did not create separate models for each DR category or split the data by disease stage. The purpose of this study was to evaluate changes in the peripapillary retinal microvasculature and retinal nerve fiber layer (RNFL) in diabetic participants across various stages of DR, and to further investigate the relationship between the peripapillary microvascular index and the RNFL.

In Table 2, which presents the linear regression of peripapillary retinal microcirculation and RNFL with various stages of DR, the outcome variables (dependent variable) include each peripapillary OCTA parameter and RNFL thickness. The phrases "Non-DR vs. Mild DR", "Non-DR vs. Moderate DR" , and "Non-DR vs. Severe DR" are included to clarify that "Non-DR" serves as the reference category (dummy variable).

As mentioned in my previous comment, multiple regression models have been fitted to different subsets of the data. Additionally, many statistical tests for trend have been conducted. This will increase the probability of a type I error occurring. If no adjustment for multiple testing is made, this should at least be mentioned as a

limitation of the study.
[Response] Thank you for highlighting this limitation. We agreed that multiple testing can potentially increase the probability of a type I error and we have included this concern as a limitation in the discussion section on page 15, line 50-52. No it reads: ***Forth, the probability of a type I error would increase because multiple statistical tests were performed.***

Additionally, the P for trend analysis in Table 2 was added during the first revision at the request of the reviewers. We agreed with you that this could increase the likelihood of a type I error and it was not necessary for Table 2. Therefore, we have removed it from the Table 2 and deleted the relative information in the manuscript accordingly, please check it.

No sample size calculation is mentioned.
[Response] This cross-sectional study is part of the Guangzhou Diabetic Eye Study (GDES), an ongoing community-based prospective cohort study conducted at the Zhongshan Ophthalmic Center, Sun Yat-sen University in Guangzhou and serves as the primary provider of eye health services in the city. The surrounding communities have a stable population and have established long-term collaborations with us, along with a registry system for diabetic patients. The detailed methodology, including data collection and sample size calculation, has been described previously.[1] In brief, type 2 diabetic patients registered in the communities near the Zhongshan Ophthalmic Center were consecutively recruited. The number of participants significantly exceeded the required sample size. For further details, please refer to the methodological article. [1]
Reference
1. Zhang S, Chen Y, Wang L, Li Y, Tang X, Liang X, He M, Wenyong H, Wang W; GDES group. Design and Baseline Data of the Diabetes Registration Study: Guangzhou Diabetic Eye Study. Curr Eye Res. 2023 Jun;48(6):591-599.

Please include in the methods section which parameters were included in the regression models.
[Response] Thank you for your valuable suggestion. We have added more detailed information regarding parameters included in the regression models in the methods section on page 9, line 60 and page 10, line 1-25. The revised text now reads: ***Univariable and multivariable linear regression analyses were used to evaluate the associations of each peripapillary OCTA parameters (VD, VLD, and VDI) and RNFL thickness with various stages of DR after adjusting for age, sex, duration of diabetes, HbA1C level, body mass index, systolic blood pressure, total cholesterol level, axial length, intraocular pressure and OCTA signal strength intensity. Furthermore, linear regression analyses were performed to assess the associations of average RNFL thickness and peripapillary OCTA parameters (VD, VLD, and VDI) after adjusting for age, sex, duration of diabetes, HbA1C, body mass index, systolic blood pressure, total cholesterol, severity of diabetic retinopathy, axial length, intraocular pressure and OCTA signal strength intensity. These confounding factors were selected based findings from the Guangzhou Diabetic Eye Study and other relevant studies.***

Which median values were compared using the test for trend? Are these the median values for each parameter in each subgroup of patients presented in Table 2? This is

unclear from the methods section.
[Response] The P for trend analysis in Table 2 was added during the first revision at the request of the reviewers. However, upon further consideration, we determined that including this analysis may increase the likelihood of a type I error and is not necessary for Table 2. As a result, we have removed it from Table 2 and updated the manuscript accordingly.

There is no mention of multicollinearity throughout and how this many affect the results and the conclusions that can be drawn from the results. For example, are the whole image, peripapillary ring, and the entire peripapillary area not likely to be correlated? Or have separate regression models been generated for each parameter, rather than including all parameters in one model? This is unclear.
[Response] Thank you for your valuable comment. Each parameter (VD, VLD, and VDI) for the whole image, the peripapillary ring, and the entire peripapillary area was analyzed separately in regression models. Therefore, these parameters were not combined into a single regression model, eliminating the need to consider multicollinearity issues.

Two sets of multivariable regression models have been presented in Tables 2 and 3. Are the models in Table 3 fitted to the whole dataset? This is unclear and is not described in the methods section. Why is RNFL included in Table 2 as a 'parameter'? The methods section suggests that this is the outcome of the model.
[Response] Thank you for your thoughtful comment. We have added more detailed information regarding linear regression models in the methods section and the models in Table 3 fitted to the whole dataset. In Table 2, linear regression analysis was used to analyze the relationship between parameters, including peripapillary retinal microvasculature and RNFL, and the severity of diabetic retinopathy, and the outcome variables (dependent variable) include each peripapillary OCTA parameter and RNFL thickness. In Table 3, linear regression analysis was used to analyze the relationship between average RNFL thickness and peripapillary OCTA parameters, and the outcome variables (dependent variable) include average RNFL thickness. Thus, RNFL is an outcome of the two models rather than a "parameter". We appreciated that you point out that the word "parameter" here is inappropriate and we have deleted it from the tables. We apologize for any misrepresentation in my article.

Including 'Non-DR vs. Mild DR' in Table 2 is confusing. The reader may interpret this as a logistic regression model comparing the peripapillary retinal microcirculation in patients with and without diabetic retinopathy. However as mentioned above, since RNFL is included as covariate in the model I question whether results in Table 2 are from a logistic regression model, using diabetic retinopathy status as the outcome. The results described on page 11 also suggest that a logistic regression model may have been used.
[Response] We sincerely apologized for any confusion caused by the tables and our descriptions. In the current study, linear regression models rather than logistic regression models were conducted in Table 2 and Table 3. The phrases "Non-DR vs. Mild DR", "Non-DR vs. Moderate DR", and "Non-DR vs. Severe DR" in Table 2 were included to clarify that "Non-DR" serves as the reference category (dummy variable) for moderate and severe DR. We apologized that we carelessly wrote "linear regression models" as "logistic regression models" in the result section on

page 11. At present, we have revised the result section to explicitly state that linear regression was used in Table 2, aligning it with the statistical methods using linear regression described in the methods sections. We acknowledge this error in the results section and deeply regret any misunderstanding it may have caused.

Minor comments

It is unclear from the abstract that RNFL thickness is the outcome in the regression models. Or as mentioned in my previous comments, perhaps the outcome for some of the models was diabetic retinopathy stage?
[Response] Thank you very much for your valuable comment. We would like to clarify that RNFL thickness is indeed the outcome (dependent variable) in the regression models. We apologize for the misdescription in the abstract, and we have made the necessary modifications on page 3, line 33-39 and page 51-55.Now it reads:

Linear regression analyses were used to evaluate the association of the peripapillary OCTA parameters (VD, VLD, and VDI), RNFL thickness with various DR stages, as well as average RNFL thickness with peripapillary OCTA parameters.

Moderate (β = -4.56, 95%CI=-8.97 to -0.15, P = 0.043) and severe DR (β = -10.12, 95%CI=-18.29 to -1.95, P = 0.015) had significant thinner peripapillary RNFL in the inferior quadrant.

How were patients sampled; were they sampled consecutively or randomly?
[Response] This cross-sectional study was part of the Guangzhou Diabetic Eye Study (GDES), an ongoing community-based prospective cohort study at the Zhongshan Ophthalmic Center, Guangzhou, China. The detailed methodology, including data collection and sample size calculation, has been described previously.[1] In brief, type 2 diabetic patients registered in the communities near the Zhongshan Ophthalmic Center, which is affiliated with Sun Yat-sen University in Guangzhou and is the primary provider for eye health service in the city, were consecutively recruited. The nearby communities have a stable population and have established long-term collaborations with us, along with a registry system for diabetic patients. The number of participants significantly exceeded the required sample size. For details, please refer to the methodological article. [1]

Reference
1. Zhang S, Chen Y, Wang L, Li Y, Tang X, Liang X, He M, Wenyong H, Wang W; GDES group. Design and Baseline Data of the Diabetes Registration Study: Guangzhou Diabetic Eye Study. Curr Eye Res. 2023 Jun;48(6):591-599.

What is 'systemic' information?
[Response] "Systemic information" refers to data that relates to the entire body or a system-wide process, rather than being localized to a specific organ, tissue, or area. In the current study, in addition to ocular information, we also collected systematic information from participants, including age, sex, duration of DM, medical and surgery history, height, weight, blood pressure, and results from venous blood test.

In Figure 1 it is apparent that 195 participants were excluded due to unqualified imaging data. Did the authors investigate whether the characteristics of these patients differed significantly to the rest of the cohort?
[Response] Thank you for your valuable comments. We agree that it was important for the characteristics of the included and excluded participants to be comparable. We conducted a comparison between the two groups, and the results showed that there were no significant differences in basic characteristics (age, gender, duration of DM, HaA1c, BMI, SBP, DBP, serum lipids and AL) between the 1,325 included participants with the 195 excluded participants.

Perhaps the table of patient characteristics could be included in the main manuscript rather than the supplementary material.
[Response] Thank you for your suggestion. The table of patient characteristics has been moved from the supplementary material to the main manuscript and is now presented as Table 1. Additionally, the numbering of other tables have been updated accordingly in both the manuscript and the tables. Please take a moment to review it.

The Kolmogorov–Smirnov test can be too conservative with low power to detect skewed distributions. Instead, the distribution of a variable can be assessed from inspection of a histogram. Also, the normality should be assessed within each group (i.e. those with/without diabetic retinopathy). If the normality assumption is not met, a non-parametric test can be used.
[Response] Thank you very much for your valuable advice, we have learned a lot. We reviewed our data, and the OCTA parameters (VD, VLD, and VDI) and the RNFL thickness in the current study were found to be normally distributed, as confirmed by the Kolmogorov–Smirnov test and histogram.

Which RNFL covariate was used as the outcome for the regression model presented in Table 3 (average, S, N, I, T)?
[Response] The outcome variable (dependent variable) for the regression model presented in Table 3 was the average RNFL. We have clarified the title of Table 3 to reflect this. Please take a moment to review it.

The study is described as a 'large cohort study' in the discussion. This is misleading since the study was cross-sectional.
[Response] I apologize for the misleading wording. We have revised it to read "This study with a large-scale of Chinese diabetic patients." Please review the changes.

How accurate is the OCT imaging and image analysis software? Perhaps this should be mentioned in the discussion, potentially as a limitation.
[Response] Thank you very much for your suggestion. We have added a discussion on the accuracy of OCT imaging and its analysis software, highlighting the potential for measurement errors as a limitation on page 15, line 52-60. Now it reads:
Fifth, although great efforts have been made to ensure the accuracy of the OCTA measurements, it is still potentially affected by systemic resolution, image artifacts and motion artifacts which cannot be completely eliminated.[1,2] Additionally, slow or stagnant blood flow may not be detected, leading to misestimation of feature analysis.[3]
Reference
1. Lu Y, Wang JC, Cui Y, Zhu Y, Zeng R, Lu ES, Katz R, Husain D, Vavvas DG, Kim

LA, Miller JW, Miller JB. A quantitative comparison of four optical coherence tomography angiography devices in healthy eyes. Graefes Arch Clin Exp Ophthalmol. 2021 Jun;259(6):1493-1501.
2. Spaide RF, Fujimoto JG, Waheed NK. IMAGE ARTIFACTS IN OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY. Retina. 2015 Nov;35(11):2163-80.
3. Spaide RF, Klancnik JM Jr, Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. JAMA Ophthalmol. 2015 Jan;133(1):45-50.