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ARTICLE

Retinal vascular tortuosity in persons with diabetes and diabetic retinopathy

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Abstract
Aim/hypothesis The aim of this hypothesis was to examine the association of retinal vessel tortuosity with diabetes and diabetic retinopathy (DR).
Methods A clinic-based study of 327 participants (224 with diabetes and 103 non-diabetic controls) aged ≥18 years. DR was graded from fundus photographs according to the modified Airlie House Classification system and categorised into mild non-proliferative DR (NPDR), moderate NPDR and vision-threatening DR (VTDR). Retinal vessel tortuosity was measured from disc-centred retinal photographs. Measurements were taken, using a semi-automated computer program by a single grader, of arterioles and venules within 0.5 to 2 disc diameters away from the optic disc.
Results There were 114 (44%) participants with DR. In the multivariate analysis, retinal arteriolar and venular tortuosity were increased in participants with diabetes without DR (mean difference 12.4×10^{-5} and 13.3×10^{-5}, respectively; both $p<0.05$) and in those with DR (mean difference 15.4×10^{-5} and 15.0×10^{-5}, respectively; both $p<0.01$) compared with non-diabetic participants. Among participants with diabetes, increased arteriolar tortuosity was significantly associated with mild NPDR (OR 1.53, 95% CI 1.03–2.05, per SD increase in arteriolar tortuosity) and moderate NPDR (OR 1.67, 95% CI 1.10–2.55) but not VTDR (OR 0.91, 95% CI 0.54–1.54). No association with DR was found for venular tortuosity.
Conclusions/interpretation Persons with diabetes had more tortuous retinal vasculature than persons without diabetes. In persons with diabetes, increased arteriolar tortuosity was associated with mild and moderate stages of DR. This suggests that retinal vascular tortuosity might be an early indicator of microvascular damage in diabetes; thus, further investigation is indicated.

Keywords Diabetes · Diabetes microvascular complications · Diabetic retinopathy · Retinal imaging · Retinal vascular tortuosity

Abbreviations

CSMO	Clinically significant macular oedema
DBP	Diastolic blood pressure
DR	Diabetic retinopathy
GEE	Generalised estimating equation
NPDR	Non-proliferative diabetic retinopathy

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SBP Systolic blood pressure
SIVA Singapore I vessel assessment
VEGF Vascular endothelial growth factor
VTDR Vision-threatening diabetic retinopathy

Introduction

Diabetic retinopathy (DR) is one of the most common complications of diabetes [1]. Although the pathogenesis of DR remains largely unknown, it has been known to be associated with duration of diabetes and increased HbA_{1c} levels [2]. Yet recent evidence suggests that these long-established risk factors explain only a proportion of risk variation of DR [3]. Identifying additional risk markers beyond these known factors is critically important to understand the pathogenesis of DR and to better manage diabetes and DR.

The retinal microvasculature provides a unique opportunity to directly and non-invasively visualise the circulatory system. A number of studies have suggested that changes in the retinal vasculature (e.g. vessel calibre, fractal dimension) might be markers of diabetes [4], diabetic retinopathy [5, 6] and nephropathy [7–9]. In addition, retinal vessel tortuosity [10] has been shown to be sensitive to diabetes-related haemodynamic changes [11], including disturbed blood flow, endothelial dysfunction [12–14], and an increased production of vascular endothelial growth factor (VEGF) [15, 16]. We recently reported that increased retinal arteriolar tortuosity was related to a high HbA_{1c} level in young patients with type 1 diabetes, including those without DR [17]. Previous experimental and clinical studies also showed that tortuous vessels were associated with DR [11, 18]. However, whether this increased retinal vessel tortuosity is present in persons with diabetes prior to the development of complications, and to what extent it may distinguish those at high risk of DR, remains unclear.

In this study, we aimed to examine the associations between retinal vessel tortuosity, measured quantitatively using a computer program, and diabetes and DR in typical diabetic patients seen in the clinics.

Methods

Study population We recruited 224 white individuals with diabetes (85 with type 1 and 139 with type 2) and 103 non-diabetic white individuals as controls between October 2006 and April 2008. Participants were consecutively recruited from the diabetes eye clinics at the International Diabetes Institute, Melbourne, VIC, Australia (diabetic participants) and from the general eye clinics at the Royal Victorian Eye and Ear Hospital (non-diabetic participants). We included patients aged 18–70 years and excluded individuals if they had a history of epilepsy or glaucoma, had undergone previous vitreous/retinal surgery and/or had a cataract on examination, or if they had any retinal or eye pathologies (except for those who had DR). For non-diabetic participants, we ensured that they had a negative past history of diabetes, confirmed with fasting glucose <7.0 mmol/l (126 mg/dl), and no previous history of diabetes-related medications (e.g. glucose-lowering drugs). The study followed the tenets of the Declaration of Helsinki, was granted ethics committee approval, and we obtained written informed consent from each participant.

Assessment of other risk factors All participants underwent a face-to-face interview using questionnaires covering past medical history, smoking status and the past and concurrent use of medications (insulin and oral glucose-lowering, anti-hypertensive and lipid-lowering medications). Fasting blood glucose level, cholesterol and triacylglycerol levels, and HbA_{1c} were examined at suburban pathology centres using fasting blood samples obtained within 2 weeks of particpants' eye examinations. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg, diastolic blood pressure (DBP) >90 mmHg, or current use of anti-hypertensive medications. Dyslipidaemia was defined as total cholesterol level >5.5 mmol/l, triacylglycerol >2.0 mmol/l or current use of lipid-lowering medications. BMI was calculated from weight (kg) divided by square height (m^2).

Assessment of DR Clinical examinations and retinal photography were performed for all participants. DR was graded from digital retinal photographs at the Retinal Vascular Imaging Centre, Centre for Eye Research, Australia, by graders who were masked to participants' clinical details. DR severity score was assigned for each eye according to the modified Airlie House Classification system [19]. DR was classified as follows: 'No DR' if the level was 10 or 12; 'mild non-proliferative DR (NPDR)' if 14–20; 'moderate NPDR' if 31 or 41; and 'severe NPDR and proliferative DR (PDR)' if it was 51–80. 'Any DR' was defined to include levels 14–80. Macular oedema was defined as present or absent, and further classified into clinically significant macular oedema (CSMO) or not. Vision-threatening DR (VTDR) was defined to include severe NPDR, PDR and CSMO.

Measurement of retinal vessel tortuosity Retinal vessel tortuosity, or curvature of vessel course, was quantificatively measured from digital disc-centred retinal photographs (field 1 of the Early Treatment Diabetic Retinopathy Study [ETDRS] photographic fields), using

a semi-automated computer program (Singapore I vessel assessment [SIVA]). Detailed measurement procedures have been previously described by Sasongko et al. and Koh et al. [17, 20]. In brief, a trained grader who was masked to participants' characteristics applied SIVA to each image. A grid, after appropriate identification of the centre of the optic disc, divided the image into three concentric zones (zone A [within 0.5 disc diameters away from the optic disc margin]; zone B [between 0.5 and 1 disc diameters away]; and zone C [between 1 and 2 disc diameters away]). All vessels within these zones were traced and identified as either an arteriole or a venule. The grader carefully checked whether all arterioles and venules were correctly identified, based on information of their parent vessels, crossing between arterioles and venules and the colour of the vessels. Corrections were made when necessary.

All vessels of more than 40μm width and routing through all three zones were measured. The averages of measures from the biggest six arterioles and six venules were summarised as tortuosity indices of arterioles and venules, respectively. We considered images to be poor quality if they were blurred or contained incomplete representation of all zones, and to be ungradable if there were fewer than four large arterioles or venules gradable in one image. Vessel tortuosity index was calculated using the integral of the total squared curvature along the path of the vessel divided by the total length of the vessel arc [10].

Statistical analysis Student's *t* test, Mann–Whitney *U* test and the χ^2 test were used to assess differences in means or proportions for participants' characteristics between individuals with and without diabetes, and between those with diabetes with and without DR. Retinal arteriolar or venular tortuosity was analysed continuously (per SD change). In the generalised estimating equation (GEE) models, we analysed retinal vascular tortuosity as a dependent variable and calculated the mean differences in tortuosity index between controls and patients with diabetes, with and without DR, and estimated the adjusted mean values of tortuosity index for each severity level of DR using eye-specific data [21]. Both retinal arteriolar and venular tortuosity were positively skewed, thus log transformation was applied. In the logistic regression model, we analysed vessel tortuosity as an independent variable, and used multinomial logistic regression models to assess the odds of having each severity level of DR associated with increasing index of arteriolar or venular tortuosity, using person-specific data, based on the eye with the worst DR level. We constructed two models: model 1 adjusted for age and sex; and model 2, which was additionally adjusted for duration of diabetes, HbA_{1c}, SBP, cholesterol, BMI, use of insulin and other medications, and vessel calibre. All statistical procedures were performed using Intercooled STATA 10.1 for Windows (StataCorp, College Station, TX, USA).

Results

The summary of participants' characteristics is shown in Table 1. There were 224 (68.5%) patients with diabetes and 144 (44%) with DR. Persons with diabetes were older, had higher BMI and cholesterol level, and were more likely to have hypertension compared with those without diabetes. Among diabetic persons, those with DR had longer durations of diabetes, higher HbA_{1c} and SBP levels, and were more likely to use insulin compared with those without DR. Mean arteriolar and venular tortuosity values were higher in persons with diabetes compared with those without. However, among those with diabetes, only arteriolar tortuosity was higher in persons with DR compared with those without (Table 1).

Table 2 shows the adjusted mean difference of tortuosity in diabetes, DR and by DR severity level. In the multivariable-adjusted model, compared with non-diabetic controls, both arteriolar and venular tortuosity indices were greater in those with diabetes but no DR (mean difference 12.4×10^{-5} and 13.3×10^{-5}, respectively; both $p<0.05$) and in those with DR (mean difference 15.4×10^{-5} and 15.0×10^{-5}, respectively; both $p<0.01$). Compared with persons with diabetes but no DR, arteriolar tortuosity was increased in mild (mean difference 2.09×10^{-5}) and moderate NPDR (mean difference 6.17×10^{-5}; Table 2). Figure 1 shows that arteriolar tortuosity increased with increasing severity of DR but subsequently decreased non-significantly in VTDR among persons with diabetes. No significant difference was found for venular tortuosity and DR severity levels (Table 2).

Among persons with diabetes, after adjusting for age, sex, duration of diabetes, HbA_{1c}, blood pressure, BMI, cholesterol, triacylglycerol, use of insulin, anti-hypertensive and lipid-lowering medications, and vessel calibre, each SD increase in arteriolar tortuosity was significantly associated with mild and moderate NPDR (OR 1.53 and 1.67, respectively; both $p<0.05$; Table 3). Associations were largely similar in analysis stratified by type 1 and type 2 diabetes status, although these associations were only of borderline significance (electronic supplementary material [ESM] Tables 1 and 2).

Discussion

In this clinical study, we showed that persons with diabetes were more likely to have tortuous arterioles and venules

Table 1 Comparisons of characteristics between participants with and without diabetes and, among those with diabetes, between patients with and without DR

Characteristic	Non-diabetic controls (n=103)	Participants with diabetes (n=224)	p value[a]	No DR (n=80)	Any DR (n=144)	p value[b]
Sex (% male)	41.3	40.9	0.92	55.8	62.0	0.35
Current cigarette smoker (%)	56.3	51.9	0.09	58.1	50.4	0.11
Hypertension (%)	20.3	60.1	<0.001	42.1	63.8	0.006
Dyslipidaemia (%)	10.9	52.3	<0.001	44.1	49.9	0.46
Insulin use (%)	–	52.4		57.9	71.3	0.037
Use of oral glucose-lowering agent (%)	–	62.6		53.7	55.8	0.75
Diabetes type (% type 1)	–	37.9		38.9	37.2	0.79
Age (years)[c]	45.5±30	59±15	<0.001	58±18	60±14	0.43
BMI (kg/m^2)	26.2±5.5	30.1±6.2	<0.001	29.5±5.6	31.1±6.6	0.07
Duration of diabetes (years)[c]	–	15±12		10±11	18±13	<0.001
HbA_{1c} (%)	–	7.8±1.5		7.6±1.7	8.0±1.2	0.022
HbA_{1c} (mmol/mol)	–	48.4±9.3		47.1±10.5	49.6±7.4	
Systolic BP (mmHg)	128.2±15.0	130.5±13.5	0.069	125.2±13.1	130.1±15.1	0.011
Cholesterol (mmol/l)	5.4±1.0	4.6±1.1	<0.001	4.7±1.1	4.5±1.1	0.47
Triacylglycerol (mmol/l)[c]	1.5±0.8	1.6±1.1	0.66	1.3±1.0	1.3±1.1	0.50
Glucose (mmol/l)	4.9±0.7	9.0±3.7	<0.001	8.2±3.5	9.6±3.8	<0.001
Vessel calibre (μm)						
Arteriolar	146.0±16.1	149.8±16.9	0.007	148.0±16.0	150.8±16.9	0.51
Venular	220.7±25.1	222.2±24.0	0.52	217.4±24.9	223.7±27.3	0.014
Vessel tortuosity (×10^5)						
Arteriolar	18.5±8.09	34.4±18.6	<0.001	33.6±16.8	37.2±21.9	0.043
Venular	22.2±9.15	39.7±18.8	<0.001	39.1±19.2	40.2±18.5	0.57

Data are mean±SD unless otherwise indicated

p values were obtained using the χ^2 test (categorical), Student's t test (continuous and normally distributed) or Mann–Whitney U test (continuous and skewed)

[a] Comparing diabetic participants with healthy controls

[b] Comparing diabetic participants with and without retinopathy

[c] Results are shown as median (IQR)

than non-diabetic controls. Among patients with diabetes, increased arteriolar tortuosity was associated with mild and moderate levels of DR, but not VTDR (severe or proliferative DR). Venular tortuosity was not associated with DR or DR severity.

Relationships between retinal vessel tortuosity and diabetes and DR have been previously demonstrated in studies in animals [18] and humans [11]. An association between retinal vessel tortuosity and diabetes and diabetic-like retinopathy was demonstrated in an experimental study of a galactose-fed rat model [18]. Kristinsson and associates also showed that patients with DR and diabetic macular oedema had elongated and more tortuous retinal vessels [11]. Although tortuosity measurement methods differed between this study by Kristinsson and our current analysis, similar associations were observed, suggesting that persons with diabetes are more likely to have tortuous retinal vessels, even in the absence of DR.

The hypothesis for the associations between abnormalities in retinal vessel tortuosity and diabetes is biologically plausible. There have been studies suggesting that increased vessel tortuosity is related to diabetes-driven haemodynamic changes such as disturbed blood flow [11, 22], tissue hypoxia [13], endothelial dysfunction [12, 23], and increased levels of VEGF [15, 16, 24]. In persons with diabetes, hyperglycaemia-mediated hypoperfusion can initiate blood flow disturbance together with the loss of endothelium cells and pericytes of the vessel wall, leading to the loss of autoregulatory function of the vessels including its compensatory mechanisms for the fluctuating hydrostatic pressure [25]. This impaired autoregulation of blood vessels could result in an undermined basement membrane and the failure to maintain the stability of the vessel wall against irregular longitudinal traction and transmural pressure [26, 27]. Subsequently, vessels become dilated and tortuous. In addition, tortuous vessels could also

Table 2 Mean differences of retinal vessel tortuosity between non-diabetic and diabetes groups and, among patients with diabetes, between those with and without diabetes retinopathy and across the severity levels of diabetic retinopathy

Group	Eyes	Mean tortuosity	Age- and sex-adjusted		Multivariable-adjusted[a,b]	
			Mean difference (95% CI)	*p* value	Mean difference (95% CI)	*p* value
Arteriolar tortuosity						
Non-diabetic controls	206	18.5	Reference		Reference	
Diabetic participants	448					
No DR	186	33.6	18.3 (12.1, 24.6)	<0.001	12.4 (2.82, 22.1)	0.011
Any DR	262	37.2	22.6 (18.1, 27.2)	<0.001	15.4 (4.5, 26.3)	0.006
Diabetic participants						
No DR	186	33.6	Reference		Reference	
Mild NPDR	60	36.8	4.41 (2.52, 6.32)	<0.001	2.09 (0.15, 4.33)	0.038
Moderate NPDR	100	46.6	12.6 (3.71, 21.4)	0.005	6.17 (0.99, 11.4)	0.019
VTDR	102	31.6	−0.09 (−1.70, 1.51)	0.91	−1.05 (−1.77, −0.32)	0.005
Venular tortuosity						
Non-diabetic controls	206	22.2	Reference		Reference	
Diabetic participants	448					
No DR	168	39.1	16.4 (9.15–23.6)	<0.001	13.3 (12.3–14.4)	<0.001
Any DR	262	40.2	17.3 (14.9–19.8)	<0.001	15.0 (10.3–19.9)	<0.001
Diabetic participants						
No DR	186	39.1	Reference		Reference	
Mild NPDR	60	39.4	0.09 (−8.61, 8.79)	0.98	1.37 (−7.07, 9.81)	0.75
Moderate NPDR	100	40.2	1.65 (0.05, 2.78)	0.05	4.14 (−0.10, 8.28)	0.056
VTDR	102	39.9	−0.70 (−7.10, 5.70)	0.83	−0.79 (−9.10, 7.52)	0.85

Data are mean tortuosity index or mean differences ($\times 10^5$) between the study group and the reference group, with *p* values estimated using GEE and eye-specific data

[a] Adjustment for age, sex, blood pressure, BMI, cholesterol, triacylglycerol, anti-hypertensive and lipid-lowering medications, and vessel calibre for mean differences between diabetes participants compared with non-diabetic controls

[b] Adjustment for age, sex, duration of diabetes, HbA_{1c}, blood pressure, BMI, cholesterol, triacylglycerol, use of insulin, anti-hypertensive, and lipid-lowering medications, and vessel calibre for mean differences among diabetic participants with and without DR

be a result of increased VEGF production in response to local tissue hypoxia and ischaemia, typically occurring in diabetes [25, 28, 29]. Although the exact mechanisms are unclear, evidence suggests that increased VEGF might induce morphological changes in the vessel, including tortuosity [15]. More importantly, tortuous vessels signify the lack of support from cells of the vessel wall, which may reflect increased fragility and vulnerability to haemorrhage [30], which appear as retinopathy signs.

In line with this, our study findings demonstrated that increased arteriolar tortuosity was associated with DR. This association was only present in mild and moderate levels of DR but not VTDR. The lack of association with VTDR might be influenced by laser treatment in those with the more severe level of DR given that laser treatment could affect overall vessel structure through atrophy and scar formation of local retinal tissues. However, after excluding 30% of eyes with VTDR which had previous laser treatment, the findings remained unchanged (ESM Tables 3 and 4). This suggests that laser treatment does not explain our observation that the severe level of DR was associated with less tortuous retinal vasculature. Our data are consistent with a previous study of retinal vascular calibre in type 2 diabetes, in which milder stages of DR were associated with arteriolar calibre widening, but more severe stages of DR were associated with arteriolar narrowing (ESM Table 5) [31]. It is conceivable that increased transmural pressure at the initial stages of DR was subsequently reduced at the severe/proliferative stages, and this may explain the reduction of vessel tortuosity and calibre in VTDR observed in this study [32]. Consistent with this, our earlier report from the same study sample showed that flickering light-induced arteriolar vasodilatation was substantially reduced in early to moderate DR cases but not so in VTDR cases [33]. In addition, it is also possible that less tortuous arteriolar vasculature seen in VTDR may be a result of the sclerotic process occurring in severe/proliferative DR [34, 35].

The strengths of our study are: the use of a quantitative method to measure retinal vessel tortuosity with high

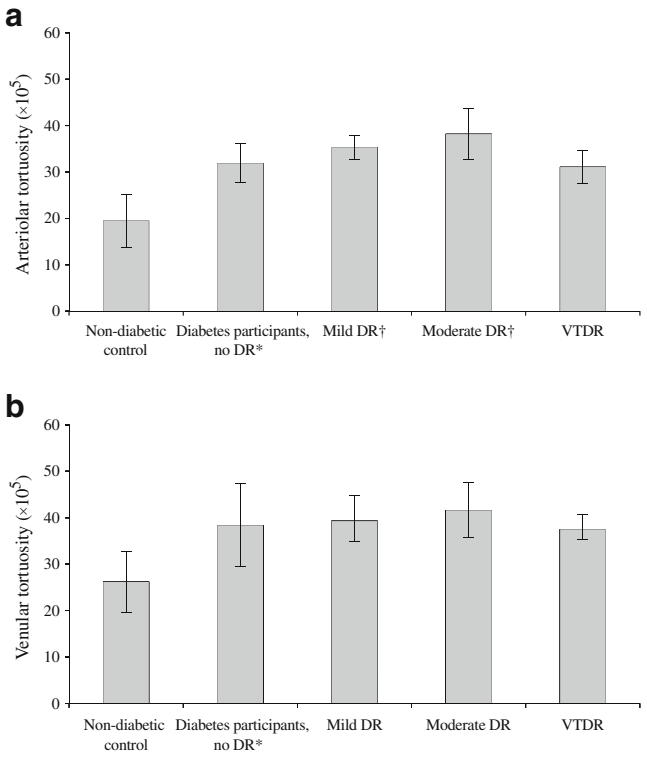

Fig. 1 Adjusted mean value (95% CI) of arteriolar (**a**) and venular (**b**) tortuosity and severity of DR. *$p<0.05$ vs non-diabetic participants; †$p<0.05$ vs diabetic participants without retinopathy

reliability, which is more accurate than qualitative assessment by an ophthalmologist as used in other studies [10]; the standardised assessment of DR from fundus photographs; and that one researcher (M. B. Sasongko) performed the tortuosity measurement masked to all participants' characteristics, thus eliminating the possibility of inter-grader variation.

However, several limitations should also be noted. First, the cross-sectional design of our study cannot provide the causality inference of the observed associations, and therefore longitudinal studies to confirm these findings are indicated. Second, as retinopathy signs were apparent during the tortuosity grading, the possibility of observer bias cannot be completely excluded. However, the grading of DR was performed long before the assessment of tortuosity, by another senior grader without knowledge of tortuosity measurement at the time of the DR grading, with adjudication from an independent retinal specialist. The measure of vessel tortuosity was performed automatically by a computer-assisted image program. Therefore, we believe that observer bias, if present, is minimal. Third, our findings might be confounded by ocular conditions associated with the normal control group recruited from eye clinics instead of the similar diabetes clinics. However, we had carefully ensured that these patients in the control group were free from diabetes and other major ocular pathologies. We have properly adjusted for participants characteristics that were significantly different (e.g. age, BMI) or that potentially confounded the analysis (e.g. blood pressure). In addition, because of the relatively small sample size, and the proportion of type 1 or type 2 diabetes being similar in those with and without DR, no distinction was made between type 1 and type 2 diabetes in the primary analysis.

We have further explored these associations in subgroups stratified by diabetes type. As the number of participants in each diabetes type was small, the subgroup findings did not reach the significance level (ESM Tables 1 and 2). Nevertheless, the pattern and direction of associations were similar between type 1 and type 2 diabetes. Finally, there was a possibility that retinal arterioles and venules were misclassified during the measurement process, particularly in participants who underwent laser treatment (4.6%), which is usually associated with poor image quality. However, reliability of our measurements were high (intra-class coefficient 0.80) [36] and our findings

Table 3 Association between retinal vessel tortuosity and severity of DR

Comparison	Age- and sex-adjusted		Multivariable-adjusted[a]	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Arteriolar tortuosity, per SD increase (3.1×10^{-5})				
vs diabetes participants, no DR				
Mild DR	1.26 (1.04–1.69)	0.012	1.53 (1.03–2.05)	0.014
Moderate DR	1.45 (1.07–1.96)	0.017	1.67 (1.10–2.55)	0.016
VTDR	0.94 (0.64–1.37)	0.75	0.91 (0.54–1.54)	0.72
Venular tortuosity, per SD increase (3.2×10^{-5})				
vs diabetes participants, no DR				
Mild DR	1.01 (0.75–1.37)	0.92	1.10 (0.79–1.55)	0.56
Moderate DR	1.09 (0.86–1.40)	0.45	1.27 (0.95–1.70)	0.10
VTDR	1.04 (0.80–1.34)	0.79	0.96 (0.69–1.33)	0.80

Data are ORs and 95% CIs for each SD increase in arteriolar or venular tortuosity associated with DR, estimated using multinomial logistic regression and person-specific data

[a] Adjustment for age, sex, duration of diabetes, HbA_{1c}, blood pressure, BMI, cholesterol, triacylglycerol, use of insulin, antihypertensive and lipid-lowering medications and vessel calibre

remained unchanged after excluding patients with previous laser treatments from the analysis (ESM Table 3).

In summary, in this study we showed the potential utility of quantitative assessment of retinal vascular tortuosity in assessing the early stage structural changes in retinal microvasculature associated with diabetes and DR. Patients with diabetes were more likely to have increased retinal vessel tortuosity than non-diabetic controls, regardless of the presence of DR. Increased arteriolar tortuosity was associated with mild to moderate DR among persons with diabetes. These findings may offer new insights into early diabetes-related microvascular changes in the retina and other end organs. Longitudinal studies are needed to determine if retinal vascular tortuosity is an early indicator of microvascular damage in diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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