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Quantitative retinal vascular features (RVFs) and cardiac biomarkers in type 2 diabetes: Findings from the PRIME cohort

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Abstract

Background

Identifying high cardiovascular (CV) risk in type 2 diabetes is critical, as cardiovascular diseases (CVD) drives most morbidity and mortality. Biomarkers such as NT-proBNP and high-sensitivity cardiac troponins detect subclinical cardiac stress and inform personalized prevention. Emerging evidence links retinal vessel calibre and microvascular features from fundus images to alterations in left ventricular structure and function. This study examined whether quantitative retinal vascular features (RVFs) in type 2 diabetes mellitus (T2DM) are associated with these cardiac biomarkers.

Methods

This study included 421 individuals with T2DM (mean age 61.17 ± 6.81 years) from the PRIME Cohort, part of The Malaysian Cohort project. All participants underwent retinal imaging and cardiac biomarker assessment, including high-sensitivity troponin T (HS-troponin) and N-terminal pro–B-type natriuretic peptide (NT-proBNP). Macula-centred retinal images were analyzed using the Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE) to evaluate RVFs, including central retinal arteriole/venule equivalent (CRAE, CRVE), arteriovenous ratio (AVR), arteriole/venule tortuosity (TORTA/V), and arteriole/venule fractal dimension (FDA/V). Incident CVD was recorded at baseline and during follow-up. Linear regression was used to analyse the relationships between RVFs and cardiac markers (HS-troponin and NTP-proBNP), while logistic regression was used to examine associations between RVFs and incident CVD.

Results

After adjusting for age and gender, AVR (adjusted β (aβ) − 0.709; 95% CI − 1.307, − 0.112; *P* = 0.020), FDA (aβ − 1.202; 95% CI − 1.849, − 0.555; *P* < 0.001), and FDV (aβ − 1.148; 95% CI − 1.786, − 0.509; *P* < 0.001) were significantly associated with HS-troponin. FDA (aβ − 26.225; 95% CI − 44.992, − 7.528; *P* = 0.006), FDV (aβ − 23.501; 95% CI − 41.956, − 5.045; *P* = 0.013), and TORTV (aβ 25.814; 95% CI 2.292, 49.337; *P* = 0.032) were significantly associated with NT-proBNP.

Conclusions

Fractal dimensions of arterioles and venules were associated with both HS-troponin and NT-proBNP, while venule tortuosity was associated only with NT-proBNP. Our study provides evidence that machine learning-derived retinal vessel features are associated with cardiac biomarkers of alterations in left ventricular structure and function.

Background

Identifying patients with type 2 diabetes who are at high risk for cardiovascular (CV) events is crucial, as cardiovascular disease remains the leading cause of morbidity and mortality in this population. Early risk stratification enables targeted preventive strategies and more intensive management. Biomarkers such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponins have emerged as valuable tools in this context. Both the American Diabetes Association (ADA) and the European Society of Cardiology (ESC) guidelines recognize these biomarkers for refining CV risk assessment in diabetes, helping clinicians detect subclinical cardiac injury or stress and guide personalized therapy to reduce future cardiovascular events [1, 2].

More recently, there is increasing evidence that retinal vessel calibre, microvascular features, and vascular characteristics derived from various imaging modalities are associated with alterations in left ventricular structure and function in stage B heart failure (HF), as well as with the incident development of symptomatic HF in the general population [3]. Additionally, machine learning applied to retinal fundus images can automatically analyze retinal vessel morphology (e.g., tortuosity, calibre, branching patterns) to enhance the detection of microvascular abnormalities [4]. We have previously shown the potential of machine learning of retinal images to predict major cardiovascular events [5, 6]. However, the relationship between machine-learning-derived retinal phenotypes and cardiac biomarkers has not been studied.

Therefore, this study explored the associations between retinal vascular features (RVFs) and two key CVD biomarkers, high-sensitivity troponin (HS-troponin) and NT-proBNP, and assessed their potential in predicting cardiovascular disease (CVD) in a large population cohort of patients with type 2 diabetes mellitus (T2DM).

Methods

Study design and participants

This study is a subset of the *PRecision Medicine for Diabetic Individuals: A joint Malaysia-UK Effort (PRIME) project*. The PRIME cohort is a collaborative initiative between the UKM Medical Molecular Biology Institute (UMBI) and the University of Dundee, United Kingdom (UK) that aims to advance precision medicine strategies for T2DM in Malaysia. This PRIME cohort comprises 5000 individuals with T2DM recruited from the Malaysian Cohort (TMC), a large population-based study involving 106,527 multiethnic Malaysian men and women aged 35–70 at baseline (2006–2012). Detailed descriptions of the TMC study design and protocol have been published previously [7]. For the current study, TMC participants with T2DM were eligible if they had completed relevant blood investigations within one month, including fasting blood glucose (FBG), glycated hemoglobin (HbA1c), fasting serum lipid (FSL), and renal profile (RP). Of the 556 participants initially recruited, 502 had complete digital macula image analyses, and 421 had valid measurements of cardiac troponin and NT-proBNP biomarkers (Fig. 1). The

aim is to quantify the retinal vascular features in T2DM and determine their association with cardiac biomarkers. This comparative cross-sectional study was conducted between 22 September 2021 and 31 July 2023 at the UKM Medical Molecular Biology Institute (UMBI) and the Hospital Canselor Tuanku Muhriz (HCTM), Universiti Kebangsaan Malaysia (UKM). All retinal imaging procedures were performed under the supervision of certified ophthalmologists.

Retinal photography

Visual acuity was assessed using a Snellen Chart (ClearChart, Reichart AMETEK, USA). Anterior segment evaluation was performed using a slit-lamp biomicroscope (Haag Streit Slit Lamp BM-900, Switzerland), to assess the risk of angle-closure glaucoma [8]. For the ocular assessment, participants received mydriatic eye drops in both eyes, using tropicamide 1% (mydriacyl, Alcon, TX, USA) and phenylephrine hydrochloride 2.5% (Mydfrin, Alcon, TX, USA) to facilitate pupillary dilatation. Topical corneal anaesthesia was administered using proparacaine hydrochloride (Alcaine; 0.5%, Alcon, TX, USA) before image capture. Standard non-stereoscopic retinal photographs were taken at a 50° field of view using a high-resolution Canon CX-1 digital fundus camera (Hybrid digital mydriatic/nonmydriatic retinal camera, USA) by a single, specially trained medical photographer.

For each participant, four images (two per eye: one optic disc-centred and one macula-centred) were acquired, with image resolutions ranging from 400 x 400 to 3,000 x 3,000 pixels. Only macula-centred images were selected for further analysis using the VAMPIRE software. These images had a standardized field of view of 45–50°, three line sizes of at least 2,000 pixels, and were saved in uncompressed, high-quality JPEG format (100%). A high-quality image was defined as one in which the vessels, optic disc, and macular region were clearly visible with good contrast [9]. All participants were also screened for incident retinopathy by an accredited medical photographer. The following exclusion criteria were applied: (i) presence of significant ocular media diseases such as cornea scar or cataract, (ii) significant retina diseases such as advanced diabetic eye disease, age-related macular degeneration, retinal detachment, or retinal scar, (iii) established diagnosis of glaucoma and on treatment, (iv) history of ocular infection and/ or inflammation within one month, and (v) those with congenital renal impairment and renal transplant, and (vi) any condition that could prevent good quality fundus photography (e.g., media opacity, inadequate pupillary dilatation or small pupil).

Retinal vascular feature measurements

Retinal vascular features (RVFs) were assessed using the Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE) software (version 3.1.0, University of Edinburgh and the University of Dundee, UK) [10, 11], based on macula-centred retinal images. Right-eye images were used for analyses unless image quality was insufficient, in which case left-eye images were substituted [12]. Seven RVF parameters were included in the analysis: central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), arteriovenous ratio (AVR), arteriolar tortuosity (TORTA), venular

tortuosity (TORTV), arteriolar fractal dimension (FDA) and venular fractal dimension (FDV). CRAE and CRVE were determined by measuring the six widest arterioles and venules crossing a standardized annular region around the optic disc, referred to as Zone B [10, 11]. AVR was calculated as the ratio of CRAE to CRVE. Tortuosity (TORTA and TORTV), indicating the degree of vessel curvature, was derived from the six widest arterioles and venules within Zone C, a concentric region further from the optic disc [13]. Fractal dimensions (FDA and FDV) representing the complexity of vascular branching were estimated through multifractal analysis using the generalized sandbox method applied to a binary vessel map from zone C [14].

Cardiac HS-Troponin and NT-proBNP Measurements

Approximately 8.5 mL of venous blood was collected from each participant using BD vacutainer® SST™ tubes. After allowing the samples to clot, they were centrifuged at 3,000 rpm for 10 minutes at room temperature. The resulting serum was aliquoted and stored at -80 ºC until analysis. High-sensitivity cardiac troponin T was measured using the Elecsys® Troponin T hs assay (REF: 08469717190), and NT-proBNP was quantified using the Elecsys proBNP II kit (REF: 08836736190). Both assays were performed on the Cobas c6000 analyzer (Roche Diagnostics, Germany) following the manufacturer's protocols for reagents and instrumentation.

Statistical analysis

All statistical analyses were conducted using SPSS software version 23.0 (IBM, USA). Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data and as median (interquartile range, IQR) for non-normally distributed data. Categorical data were reported as frequencies and percentages. Descriptive statistics were used to summarise participant demographics and clinical characteristics. Group comparisons were conducted using the independent t-test for normally distributed continuous variables, the Mann-Whitney *U* test for non-normally distributed variables (cardiac markers and incident CVD), and the chi-square test for categorical variables. For regression analyses, selected RVF parameters such as CRAE, CRVE, AVR, FDA, and FDV were standardized using z-score transformations. TORTA and TORTV values were log-transformed to normalise distribution and allow meaningful comparisons. Linear regression models were used to evaluate associations between specific RVF parameters and cardiac biomarkers (HS-troponin and NTP-proBNP), while logistic regression models assessed the relationships between RVF parameters and incident CVD. All regression analyses were adjusted for the following covariates using the Enter method: age, sex, ethnicity (Malay, Chinese, Indian), smoking status, duration of diabetes, history of CVD, medication use (anti-hypertensive and anti-diabetic drugs), body mass index (BMI), systolic blood pressure (systolic BP), FBG, HbA1c, total cholesterol, high-density lipoprotein (HDL), non-HDL cholesterol, low-density lipoprotein (LDL), triglycerides, estimated glomerular filtration rate (eGFR), and cardiac biomarkers (HS-troponin and NTP-proBNP). A statistical significance was set at $P < 0.05$.

Figure 1 Flowchart of the study selection process

Results

Demographic characteristics

A total of 421 individuals with T2DM who had complete data on RVFs and cardiac biomarkers were included in the analysis (Table 1). The mean age of participants was 61.17 ± 6.81 years, with an average diabetes duration of 10.38 ± 6.29 years. Over the 10-year follow-up period, 49 participants (11.6%) developed incident CVD, of whom 29 (59.2%) had a prior history of CVD at baseline. Participants who developed incident CVD were predominantly older, male, and of Indian ethnicity. This group also had a longer duration of diabetes and a higher reported use of anti-hypertensive and anti-diabetic medications. A slightly higher proportion of poor glycemic control was observed in the incident CVD group. Interestingly, participants with incident CVD showed significantly lower LDL and non-HDL cholesterol. No significant differences were observed between groups for HbA1c, total corrected cholesterol, HDL cholesterol, triglycerides, eGFR, BMI, or systolic BP.

Table 1 Baseline characteristics of individuals by incident CVD (n = 421)

Parameter	CVD status			
	Yes	**No**	**Total**	***P*-value**
	49 (11.6)	372 (88.4)	421 (100.0)	
Age, years	63.18 (6.74)	60.91 (6.79)	61.17 (6.81)	0.028*
Gender				0.041*
Male	33 (14.60)	193 (85.40)	226 (100.00)	
Female	16 (8.20)	179 (91.80)	195 (100.00)	
Race				<0.001*
Malay	11 (7.90)	128 (92.10)	139 (100)	
Indian	24 (23.8)	77 (76.2)	101 (100)	
Chinese	14 (7.7)	167 (92.3)	181 (100)	
Duration of diabetes, years	13.2 (9.74)	10 (5.59)	10.38 (6.29)	0.028*
Previous CVD event				<0.001*
Yes	29 (100.0)	0 (0.0)	29 (100.0)	
No	20 (5.1)	372 (94.9)	392 (100.0)	
HbA1c (%)	7.64 (1.98)	7.45 (1.99)	7.47 (1.99)	0.525
Fasting blood glucose (mmol/L)	7.67 (3.16)	8.17 (2.86)	8.11 (2.89)	0.252
Glycemic control				0.235
Poor control (≥ 7.0%)	28 (13.5)	179 (86.5)	207 (100.0)	
Good control (< 7.0%)	21 (9.8)	193 (90.2)	214 (100.0)	
Cholesterol corrected (mmol/L)	5.23 (1.20)	5.51 (1.21)	5.47 (1.21)	0.124
HDL (mmol/L)	1.27 (0.26)	1.32 (0.37)	1.32 (0.35)	0.306
LDL (mmol/L)	2.49 (0.98)	2.89 (1.12)	2.84 (1.11)	0.020*
Triglyceride (mmol/L)	1.43 (0.67)	1.57 (0.78)	1.55 (0.77)	0.218
Non-HDL	3.14 (1.06)	3.62 (1.18)	3.57 (1.17)	0.007*
eGFR	87.55 (18.03)	91.27 (15.4)	90.84 (15.75)	0.120
BMI (kg/m^2)	25.88 (3.96)	26.81 (4.38)	26.7 (4.33)	0.161
Smoking status				0.450
Yes	10 (14.3)	60 (85.7)	70 (100.0)	

No	39 (11.1)	312 (88.9)	351 (100.0)	
Retinopathy status				0.913
Yes	2 (12.5)	14 (87.5)	16 (100.0)	
No	47 (11.6)	358 (88.4)	405 (100.0)	
Medication status				
Diabetes				0.009*
Yes	40 (14.7)	233 (85.3)	273 (100.0)	
No	9 (6.1)	139 (93.9)	148 (100.0)	
Hypertension				0.020*
Yes	33 (15.1)	185 (84.9)	218 (100.0)	
No	16 (7.9)	187 (92.1)	203 (100.0)	

Data were mean (SD), median (interquartile range, IQR), or n (%). * Significance at $P < 0.05$. CVD, cardiovascular disease; HbA1c, glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; BMI, body mass index; BP, blood pressure

Table 1 (Insert here)

Cardiac markers and retinal vascular feature measurements

Both cardiac biomarkers, HS-troponin and NT-proBNP were significantly elevated in participants who developed incident CVD. HS-troponin showed a crude ratio (CR) of 1.063 (95% CI: 1.026–1.102; P = 0.001) while NT-proBNP demonstrated a CR of 1.003 (95% CI: 1.001–1.005; P = 0.001), with a near two-fold increase in median levels observed for NT-proBNP among those with incident CVD (Fig. 2).

Figure 2 Scatterplots of **(A)** HS-troponin and **(B)** NT-proBNP by CVD status. * Indicates a significant difference at $P < 0.05$

All the digital macula-centred images were successfully analyzed using the VAMPIRE software. Representative examples of these images are shown in Fig. 3. Among the RVFs, participants who developed incident CVD had significantly higher arteriole tortuosity (TORTA) compared to those without incident CVD (P = 0.047). No significant differences were observed in retinal vessel calibre (CRAE/CRVE), AVR, venule tortuosity (TORTV), or fractal dimension (FDA/V) between the two groups (Table 2).

Figure 3 Randomly selected retinal fundus images from participants without cardiovascular disease (CVD) **(A - C)** and with incident CVD **(D - F)**, analysed using the Vascular Assessment and Measurement

Platform for Images of the Retina (VAMPIRE) software version 3.1.0. In these images, venules appear as thicker and darker red, whereas arterioles are thinner and lighted red

Table 2 Summary of the retinal vascular features with incident CVD (n = 421)

Retinal vascular features	CVD status		Total	*P*-value
	Yes	No	421 (100.0)	
	49 (11.6)	372 (88.4)		
CRAE (μm)	27.251 (1.685)	27.684 (1.748)	27.633 (1.745)	0.103
CRVE (μm)	35.424 (2.389)	35.327 (2.63)	35.339 (2.601)	0.806
Log TORTA – median	-10.058 (1.028)	-10.366 (1.016)	-10.33 (1.021)	0.047*
Log TORTV – median	-9.341 (0.771)	-9.543 (0.725)	-9.519 (0.732)	0.069
AVR	0.772 (0.061)	0.787 (0.066)	0.785 (0.066)	0.133
FDA	1.352 (0.045)	1.354 (0.044)	1.354 (0.044)	0.753
FDV	1.362 (0.056)	1.366 (0.054)	1.365 (0.054)	0.703

Data were mean (SD), or n (%). * Significance at $P < 0.05$. Values for arteriole and venule tortuosity (TORTA/V) were normalized using $\log_e$ transformation. CVD, cardiovascular disease; CRAE/ CRVE, central retinal arteriole/venule equivalent; TORTA/V, arteriole/venule tortuosity; AVR, arteriovenous ratio; FDA/V, arteriole/venule fractal dimension

Associations between retinal vascular features and cardiac markers

Significant associations were identified between selected RVF parameters and the cardiac biomarkers. In unadjusted linear regression models, HS-troponin demonstrated significant inverse associations with AVR (unadjusted β (uβ) = -0.777; 95% CI: -1.432 to -0.122; $P = 0.020$), FDA (uβ = − 1.546; 95% CI: − 2.189 to − 0.904; $P < 0.001$), and FDV (uβ = -1.482; 95% CI: -2.126 to -0.838; $P < 0.001$) (Fig. 4a). These associations remained statistically significant after adjusting for age and sex (Fig. 4a): AVR (adjusted β (aβ) = − 0.709; 95% CI: − 1.307 to − 0.112; $P = 0.020$), FDA (aβ = − 1.202; 95% CI: − 1.849 to − 0.555; $P < 0.001$), and FDV (aβ = − 1.148; 95% CI: − 1.786 to − 0.509; $P < 0.001$). Regarding NT-proBNP, unadjusted models showed significant inverse associations with FDA (uβ = − 37.547; 95% CI: − 54.728 to − 20.367; $P < 0.001$) and FDV (uβ = − 34.675; 95% CI: − 51.912 to − 17.439; $P < 0.001$) (Fig. 4b). After adjustment for age and sex (Fig. 4b), significant associations persisted for FDA (aβ = − 26.225; 95% CI: − 44.992 to − 7.528; $P = 0.006$), FDV (aβ = − 23.501; 95% CI: − 41.956 to − 5.045; $P = 0.013$), with venular tortuosity (TORTV) also showing a significant positive association (aβ = 25.814; 95% CI: 2.292 to 49.337; $P =$

0.032). No significant associations were observed for the remaining RVF parameters with either HS-troponin or NT-proBNP in both unadjusted and adjusted models.

Figure 4 Forest plots for the association between retinal vascular features and cardiac markers: **(A)** HS-troponin and **(B)** NT-proBNP among type 2 diabetes participants in unadjusted β (95% CI): regression coefficient and 95% confidence interval and adjusted models (age and gender).

* Significant $P < 0.05$. Values for arteriole and venule tortuosity were normalized using log e transformation. HS-Troponin: high-sensitivity troponin, NT-proBNP: N-terminal pro b-type natriuretic peptide, CRAE/ CRVE: central retinal arteriole/venule equivalent, TORTA/V: arteriole/venule tortuosity, AVR: arteriovenous ratio, FDA/V: arteriole/venule fractal dimension

Associations between retinal vascular features and incident CVD

TORTV, AVR, FDA, and FDV were further evaluated for their association with incident CVD, given their prior significance with cardiac biomarkers. In the unadjusted logistic regression model (Table 3), TORTV showed a crude odds ratio (OR) of 1.456 (95% CI 0.970, 2.185; $P > 0.05$).AVR (OR: 0.788; 95% CI: 0.577 to 1.076; $P > 0.05$), FDA (OR: 0.954; 95% CI: 0.710 to 1.280; $P > 0.05$), and FDV (OR: 0.945; 95% CI: 0.707 to 1.263; $P > 0.05$) also showed no statistically significant associations.

After adjusting for age and sex (Table 3), the adjusted odds ratio (aOR) for TORTV remained comparable (aOR: 1.433; 95% CI: 0.953 to 2.157; $P > 0.05$), while FDA (aOR: 1.108; 95% CI: 0.792 to 1.550; $P > 0.05$) and FDV (aOR: 1.074; 95% CI: 0.775 to 1.489; $P > 0.05$) shifted above 1, indicating a potential but non-significant positive association. The inverse association for AVR persisted in the adjusted model (aOR: 0.785; 95% CI: 0.571 to 1.080; $P > 0.05$). Further adjustments of these four parameters with other confounding variables listed in Table 1 remained statistically non-significant (Supplementary Table 1).

Table 3 Association of retina vascular features with incident CVD

Variables	Univariate		Multivariate	
	OR (95% CI)	***P*-value**	**Adjusted OR (95% CI)**	***P*-value**
TORTV	1.456 (0.970, 2.185)	0.070	1.433 (0.953, 2.157)	0.084
AVR	0.788 (0.577, 1.076)	0.133	0.785 (0.571, 1.080)	0.137
FDA	0.954 (0.710, 1.280)	0.752	1.108 (0.792, 1.550)	0.550
FDV	0.945 (0.707, 1.263)	0.703	1.074 (0.775, 1.489)	0.667

Odds ratios (ORs) and 95% confidence intervals (CIs) were reported for both univariate and multivariate logistic regression analyses. Multivariate models were adjusted for age and sex. A P-value < 0.05 was considered statistically significant. The venular tortuosity (TORTV) variable was normalised using $\log_e$

transformation to meet model assumptions. The RVF parameters evaluated included TORTV (venule tortuosity), AVR (arteriovenous ratio), and fractal dimensions of arterioles (FDA) and venules (FDV)

Discussion

This study assessed the potential of RVFs in predicting CVD incidence and their relationship with cardiac biomarkers. The findings revealed that both arteriole and venule fractal dimensions were significantly associated with the cardiac biomarkers HS-troponin T and NT-proBNP. In contrast, venular tortuosity was associated only with NT-proBNP. However, none of the RVFs were significantly associated with CVD incidence. Fractal dimension (FD) reflects the branching complexity of the retinal vasculature [15, 16], while tortuosity of arterioles (TORTA) and venules (TORTV) indicates the curvature and twisting of the blood vessels, markers of microvascular remodelling linked to vascular and systemic diseases [17]. Previous studies have reported associations between FD and tortuosity with CVD complications [5, 18–23]. The significant associations between RVFs and cardiac biomarkers observed in this study suggest the potential utility of RVFs in identifying individuals with high cardiovascular risk before clinical events occur.

Several cohort studies have explored the predictive value of retinal vascular changes as predictors of CVD risk in individuals with type 2 diabetes mellitus (T2DM). The Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) study found that reduced arteriolar FD (FDA), narrower arteriolar diameter (CRAE), and increased venular tortuosity (TORTV) independently predicted 10-year major adverse cardiovascular events (MACE), offering added prognostic value beyond traditional risk scores and a coronary heart disease polygenic risk score (CHD PRS) [5]. Similarly, the Edinburgh Type 2 Diabetes Study (ET2DS) reported that increased arteriolar tortuosity and reduced FD were significantly associated with cerebrovascular events, particularly stroke (HR 1.27 and HR 0.74, respectively), even after adjusting for conventional CVD risk factors (HR 1.26 and HR 0.73) [18]. Multiple other cohorts have replicated these findings [19–23], supporting a link between retinal vasculature architecture and systemic microvascular conditions.

Notably, a recent study of deep learning of the phenomic and genomic data of 54,813 UK Biobank participants found that reduced FD was significantly associated with higher mortality among individuals with cardiometabolic conditions such as hypertension and diabetes, compared to those without [24]. The genome-wide association analysis identified multiple gene loci related to FD, many of which are involved in angiogenesis, cancer, pigmentation, inflammation, and vascular structure [24]. Although the mechanistic explanation for how reduced vascular branching and increased tortuosity contribute to the CVD risk remains limited, earlier studies suggest that diabetes-induced hypoxia, the subsequent pericyte apoptosis, and activation of the renin-angiotensin system impair cell growth and angiogenesis [25, 26], leading to reduced vessel density and a sparser vascular network, as commonly observed in diabetic individuals [27].

In the present study, reduced fractal dimension (FD) and increased TORTV were significantly associated with elevated cardiac biomarkers, but not with the incidence of CVD. This discrepancy may be explained by the possibility that the sparse vasculature and increased tortuosity restrict blood flow, which may contribute more to cerebrovascular events such as stroke rather than to other forms of CVD. This hypothesis aligns with findings from ET2DS, which showed significant associations of RVFs with stroke, but not with coronary heart disease (CHD) [18]. Nonetheless, more studies are needed to clarify these relationships, as evidence on RVFs and CVD incidence in T2DM populations remains limited.

A reduced AVR, reflecting narrower CRAE compared to CRVE, is a known predictor of CVD risk and mortality. Previous studies have linked changes in AVR to increased risk of stroke and myocardial infarction [28, 29]. The Wisconsin Epidemiologic Study of Diabetic Retinopathy found that narrower CRAE was associated with 22-year all-cause mortality, while both narrower CRAE and wider CRVE were linked to stroke mortality [30]. Similarly, the Atherosclerosis Risk in Communities (ARIC) study reported that narrower CRAE and wider CRVE predicted higher risk of death and stroke in both sexes, and CHD in women [31]. In that study, women with the narrowest CRAE had a 10-year CVD risk of 5.6% compared to 2.8% in those with the widest CRAE (2.8%). Notably, 21% of women classified as low-risk by the traditional CVD risk models were reclassified to intermediate risk based on RVFs, demonstrating their potential to enhance risk prediction, particularly in women.

This association between arteriolar narrowing and increased CVD risk has also been observed in individuals with Type 1 diabetes [32, 33], and in general population studies [22, 34]. Although previous research has not directly investigated the relationship between RVFs and cardiac biomarkers, the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study found that reduced CRAE was associated with elevated cardiac troponin T [35], a specific biomarker for cardiac injury [36], while a larger CRVE was associated with lower NT-proBNP [35] levels, a marker of cardiac stress [37]. Additionally, elevated NT-proBNP predicted the 10-year stroke risk in that study [35].

The Singaporean Epidemiology of Eye Disease (SEED) study, which followed 1770 individuals with T2DM for six years, reported that narrower arteriolar calibre preceded the onset of hypertension, while reduced arteriolar branching angle preceded both CVD and hypertension [38]. These findings suggest that retinal vascular changes may precede and potentially predict the cardiometabolic outcomes.

This study has some limitations. Only 49 of the 421 participants (11.6%) had established CVD, which may have limited the statistical power to detect associations between RVFs and CVD events. CVD results from a complex interplay of factors such as hypertension, dyslipidemia, smoking, and genetic predisposition, all of which may also influence retinal vascular changes. Additionally, microvascular alterations may take years to manifest in clinical CVD events, thus, longer follow-up is necessary to confirm the associations observed here. Future studies with larger sample sizes and extended follow-up durations are warranted to validate these findings and to further explore the predictive value of RVFs and cardiac biomarkers for cardiovascular risk in individuals with diabetes.

Conclusion

The significant associations between AVR, FDA, and FDV with HS-troponin suggest a potential link between retinal vascular narrowing and sub-clinical myocardial injury. Similarly, the associations of FDA, FDV, and venular tortuosity (TORTV) with NT-proBNP indicate that fractal alterations and increased tortuosity in the retinal vasculature may reflect underlying hemodynamic stress and early cardiac dysfunction. However, these retinal parameters were significantly associated with incident CVD, implying that while retinal microvascular changes align with cardiac stress biomarkers, they might not directly predict broad CVD outcomes. It is possible that specific CVD events, such as stroke, may be more closely related to these retinal features. Despite this, retinal imaging remains a promising adjunct to traditional risk assessment by providing early insights into vascular health. Future large-scale longitudinal studies are warranted to clarify the predictive value of these retinal markers for specific cardiovascular outcomes. Moreover, integrating retinal imaging, cardiac biomarkers and conventional clinical risk factors using AI-driven approaches may enhance the accuracy and clinical utility of CVD prediction mode.

Abbreviations

CV
Cardiovascular
RVFs
Retinal vascular features
CVD
Cardiovascular diseases
T2DM
Type 2 diabetes mellitus
PRIME
PRecision Medicine for Diabetic Individuals: A joint Malaysia-UK Effort (PRIME) project
NT-proBNP
N-terminal pro–B-type natriuretic peptide
HS-troponin
High-sensitivity troponin T
VAMPIRE
Vascular Assessment and Measurement Platform for Images of the Retina
CRAE
Central retinal arteriole equivalent
CRVE
Central retinal venule equivalent
AVR
Arteriovenous ratio
TORTA

Arteriole/venule tortuosity
TORTV
Venule tortuosity
FDA
Arteriole fractal dimension
FDV
Venule fractal dimension
ADA
American Diabetes Association
ESC
European Society of Cardiology
UKM
Universiti Kebangsaan Malaysia
UMBI
UKM Medical Molecular Biology Institute
UK
United Kingdom
TMC
The Malaysian Cohort
FBG
Fasting blood glucose
RP
Renal profile
HCTM
Hospital Canselor Tuanku Muhriz
SPSS
Statistical Package for the Social Sciences
SD
Standard deviation
BMI
Body mass index

Declarations

Ethics approval and consent to participate

Ethics approval was obtained from the UKM Research Ethics Committee (Approval no.: JEP-2019-754). All procedures were conducted in accordance with the Declaration of Helsinki and the Malaysian Guidelines for Good Clinical Practice. Written informed consent was obtained from all participants before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

NAAM and JB drafted the original manuscript. NAAM and SAS coordinated the study. SS, CP, NM, JCH, AAAR, ET, SH, EP, IRM and AD contributed to the study design and development of the research protocol. SH, MRKM, and ET analyzed the retinal vascular features using the VAMPIRE software. JCH and IK conducted retinal assessments and supervised retinal image acquisition. NA, MAK, and NAMY performed the retinal image acquisition. NAJ and NI conducted cardiac biomarker measurements. ASKA, SAS, and JB performed retinal data analysis and contributed to the manuscript editing. CCL and RJ conceived the study, provided leadership in the study design, secured funding, and performed the final editing. All authors contributed to manuscript revision and approved the final submitted version.

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Data Availability

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

References

1. Pop-Busui R, Januzzi JL, Bruemmer D, Butalia S, Green JB, Horton WB, et al. Heart failure: an underappreciated complication of diabetes. A consensus report of the American Diabetes Association. Diabetes Care. 2022;45(7):1670–90.

2. Marx N, Federici M, Schütt K, Müller-Wieland D, Ajjan RA, Antunes MJ, et al. 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes: Developed by the task force on the management of cardiovascular disease in patients with diabetes of the European Society of Cardiology (ESC). Eur Heart J. 2023;44(39):4043–140.
3. Chaikijurajai T, Ehlers JP, Tang WW. Retinal microvasculature: a potential window into heart failure prevention. Heart Fail. 2022;10(11):785–91.
4. Perez-Rovira A, MacGillivray T, Trucco E, Chin K, Zutis K, Lupascu C et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. In: 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society: 2011: IEEE; 2011: 3391–3394.
5. Mordi IR, Trucco E, Syed MG, MacGillivray T, Nar A, Huang Y, et al. Prediction of major adverse cardiovascular events from retinal, clinical, and genomic data in individuals with type 2 diabetes: a population cohort study. Diabetes Care. 2022;45(3):710–6.
6. Syed MG, Trucco E, Mookiah MR, Lang CC, McCrimmon RJ, Palmer CN, et al. Deep-learning prediction of cardiovascular outcomes from routine retinal images in individuals with type 2 diabetes. Cardiovasc Diabetol. 2025;24(1):3.
7. Jamal R, Syed Zakaria SZ, Kamaruddin MA, Abd Jalal N, Ismail N, Mohd Kamil N, et al. Cohort Profile: The Malaysian Cohort (TMC) project: a prospective study of non-communicable diseases in a multi-ethnic population. Int J Epidemiol. 2015;44(2):423–31.
8. Martin R. Cornea and anterior eye assessment with slit lamp biomicroscopy, specular microscopy, confocal microscopy, and ultrasound biomicroscopy. Indian J Ophthalmol. 2018;66(2):195–201.
9. Alais R, Dokládal P, Erginay A, Figliuzzi B, Decencière E. Fast macula detection and application to retinal image quality assessment. Biomed Signal Process Control. 2020;55:101567.
10. Trucco E, Giachetti A, Ballerini L, Relan D, Cavinato A, MacGillivray T. Morphometric measurements of the retinal vasculature in fundus images with VAMPIRE. Biomedical Image Underst. 2015:91–111.
11. Mookiah MRK, Hogg S, MacGillivray T, Trucco E. On the quantitative effects of compression of retinal fundus images on morphometric vascular measurements in VAMPIRE. Comput Methods Programs Biomed. 2021;202:105969.
12. Forster RB, Garcia ES, Sluiman AJ, Grecian SM, McLachlan S, MacGillivray TJ, et al. Retinal venular tortuosity and fractal dimension predict incident retinopathy in adults with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. Diabetologia. 2021;64:1103–12.
13. Lisowska A, Annunziata R, Loh GK, Karl D, Trucco E. An experimental assessment of five indices of retinal vessel tortuosity with the RET-TORT public dataset. In: 2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society: 2014: IEEE; 2014: 5414–5417.
14. McGrory S, Taylor AM, Pellegrini E, Ballerini L, Kirin M, Doubal FN, et al. Towards standardization of quantitative retinal vascular parameters: comparison of SIVA and VAMPIRE measurements in the Lothian Birth Cohort 1936. Transl Vis Sci Technol. 2018;7(2):12–12.

15. Azemin MC, Kumar DK, Wong TY, Kawasaki R, Mitchell P, Wang JJ. Robust methodology for fractal analysis of the retinal vasculature. IEEE Trans Med Imaging. 2010;30(2):243–50.
16. Ab Hamid F, Che Azemin MZ, Salam A, Aminuddin A, Mohd Daud N, Zahari I. Retinal vasculature fractal dimension measures vessel density. Curr Eye Res. 2016;41(6):823–31.
17. Hossain MMN, Hu NW, Kazempour A, Murfee WL, Balogh P. Hemodynamic characteristics of a tortuous microvessel using high-fidelity red blood cell resolved simulations. Microcirculation. 2024;31(7):e12875.
18. Sandoval-Garcia E, McLachlan S, Price AH, MacGillivray TJ, Strachan MW, Wilson JF, et al. Retinal arteriolar tortuosity and fractal dimension are associated with long-term cardiovascular outcomes in people with type 2 diabetes. Diabetologia. 2021;64(10):2215–27.
19. Cheung CY-l, Tay WT, Ikram MK, Ong YT, De Silva DA, Chow KY, et al. Retinal microvascular changes and risk of stroke: the Singapore Malay Eye Study. Stroke. 2013;44(9):2402–8.
20. Liew G, Gopinath B, White AJ, Burlutsky G, Yin Wong T, Mitchell P. Retinal vasculature fractal and stroke mortality. Stroke. 2021;52(4):1276–82.
21. Yusufu M, Chen Y, Dayimu A, Bulloch G, Jin S, Vingrys AJ, et al. Retinal vascular measurements and mortality risk: evidence from the UK Biobank study. Transl Vis Sci Technol. 2024;13(1):2–2.
22. Hughes AD, Falaschetti E, Witt N, Wijetunge S, Thom SAM, Tillin T, et al. Association of retinopathy and retinal microvascular abnormalities with stroke and cerebrovascular disease. Stroke. 2016;47(11):2862–4.
23. Ong Y-T, De Silva DA, Cheung CY, Chang H-M, Chen CP, Wong MC, et al. Microvascular structure and network in the retina of patients with ischemic stroke. Stroke. 2013;44(8):2121–7.
24. Zekavat SM, Raghu VK, Trinder M, Ye Y, Koyama S, Honigberg MC, et al. Deep learning of the retina enables phenome-and genome-wide analyses of the microvasculature. Circulation. 2022;145(2):134–50.
25. Nakaizumi A, Puro DG. Vulnerability of the retinal microvasculature to hypoxia: role of polyamine-regulated KATP channels. Invest Ophthalmol Vis Sci. 2011;52(13):9345–52.
26. Jarajapu YP, Bhatwadekar AD, Caballero S, Hazra S, Shenoy V, Medina R, et al. Activation of the ACE2/angiotensin-(1–7)/Mas receptor axis enhances the reparative function of dysfunctional diabetic endothelial progenitors. Diabetes. 2013;62(4):1258–69.
27. Arnould L, Binquet C, Guenancia C, Alassane S, Kawasaki R, Daien V, et al. Association between the retinal vascular network with Singapore I Vessel Assessment (SIVA) software, cardiovascular history and risk factors in the elderly: The Montrachet study, population-based study. PLoS ONE. 2018;13(4):e0194694.
28. Hanssen H, Streese L, Vilser W. Retinal vessel diameters and function in cardiovascular risk and disease. Prog Retin Eye Res. 2022;91:101095.
29. Colcombe J, Mundae R, Kaiser A, Bijon J, Modi Y. Retinal findings and cardiovascular risk: prognostic conditions, novel biomarkers, and emerging image analysis techniques. J Pers Med. 2023;13(11):1564.

30. Klein R, Klein BE, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes: XXI: the Wisconsin Epidemiologic Study of Diabetic Retinopathy. Ophthalmology. 2007;114(10):1884–92.
31. Seidelmann SB, Claggett B, Bravo PE, Gupta A, Farhad H, Klein BE, et al. Retinal vessel calibers in predicting long-term cardiovascular outcomes: the atherosclerosis risk in communities study. Circulation. 2016;134(18):1328–38.
32. Roy MS, Klein R, Janal MN. Relationship of retinal vessel caliber to cardiovascular disease and mortality in African Americans with type 1 diabetes mellitus. Arch Ophthalmol. 2012;130(5):561–7.
33. Miller RG, Prince CT, Klein R, Orchard TJ. Retinal vessel diameter and the incidence of coronary artery disease in type 1 diabetes. Am J Ophthalmol. 2009;147(4):653–60.
34. Wang JJ, Liew G, Klein R, Rochtchina E, Knudtson MD, Klein BE, et al. Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations. Eur Heart J. 2007;28(16):1984–92.
35. Wentzel A, Malan L, Smith W, Von Känel R, Malan NT. Retinal vasculature reactivity during flicker light provocation, cardiac stress and stroke risk in Africans: the SABPA study. Transl Stroke Res. 2019;10(5):485–94.
36. Garg P, Morris P, Fazlanie AL, Vijayan S, Dancso B, Dastidar AG, et al. Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. Intern Emerg Med. 2017;12(2):147–55.
37. Sakamoto D, Sotomi Y, Matsuoka Y, Nakatani D, Okada K, Sunaga A, et al. Prognostic utility and cutoff differences in NT-proBNP levels across subgroups in heart failure with preserved ejection fraction: insights from the PURSUIT-HFpEF registry. J Card Fail. 2025;31(5):771–80.
38. Betzler BK, Sabanayagam C, Tham YC, Cheung CY, Cheng CY, Wong TY, et al. Retinal vascular profile in predicting incident cardiometabolic diseases among individuals with diabetes. Microcirculation. 2022;29(4–5):e12772.

Figures

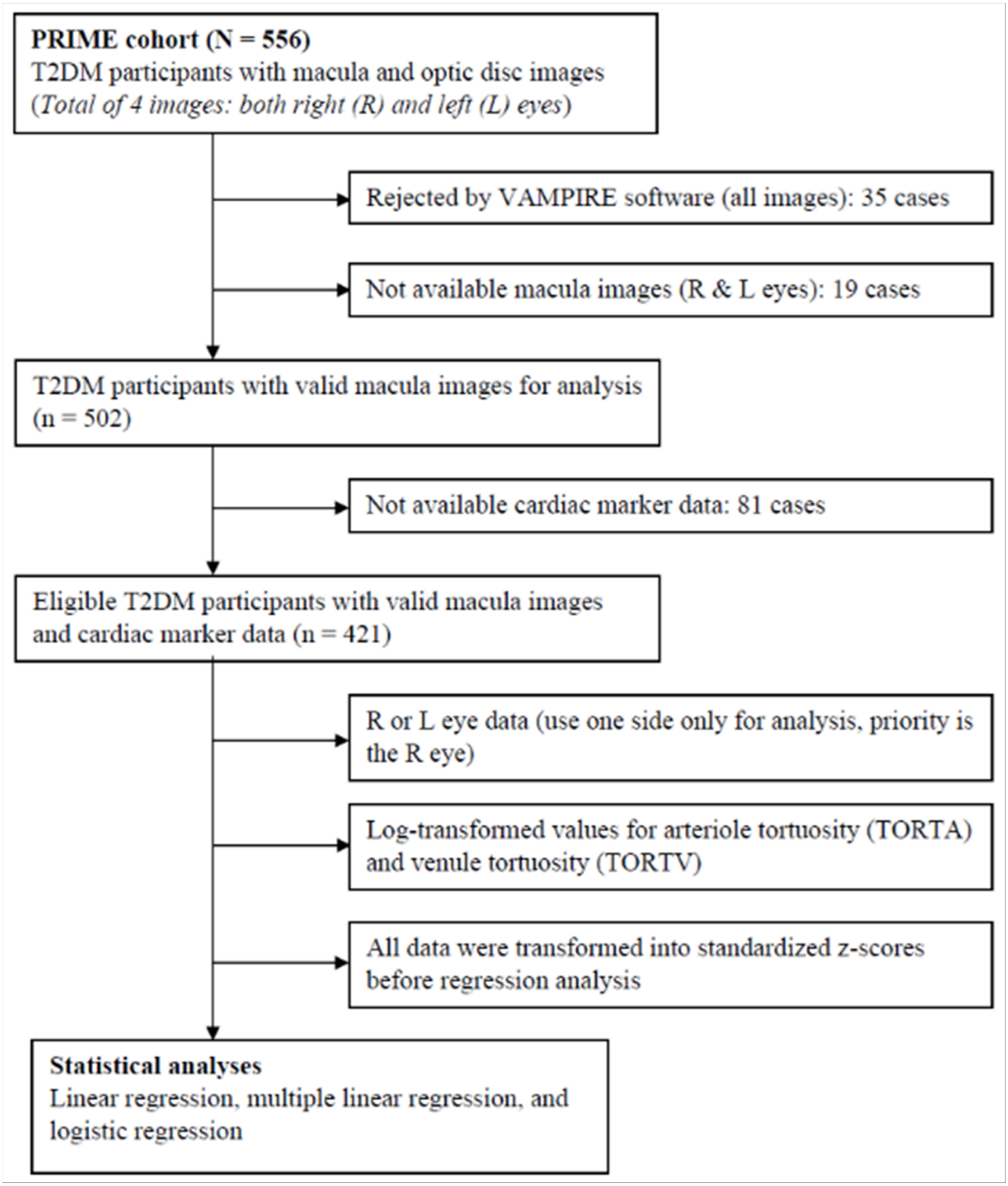


Figure 1

Flowchart of the study selection process

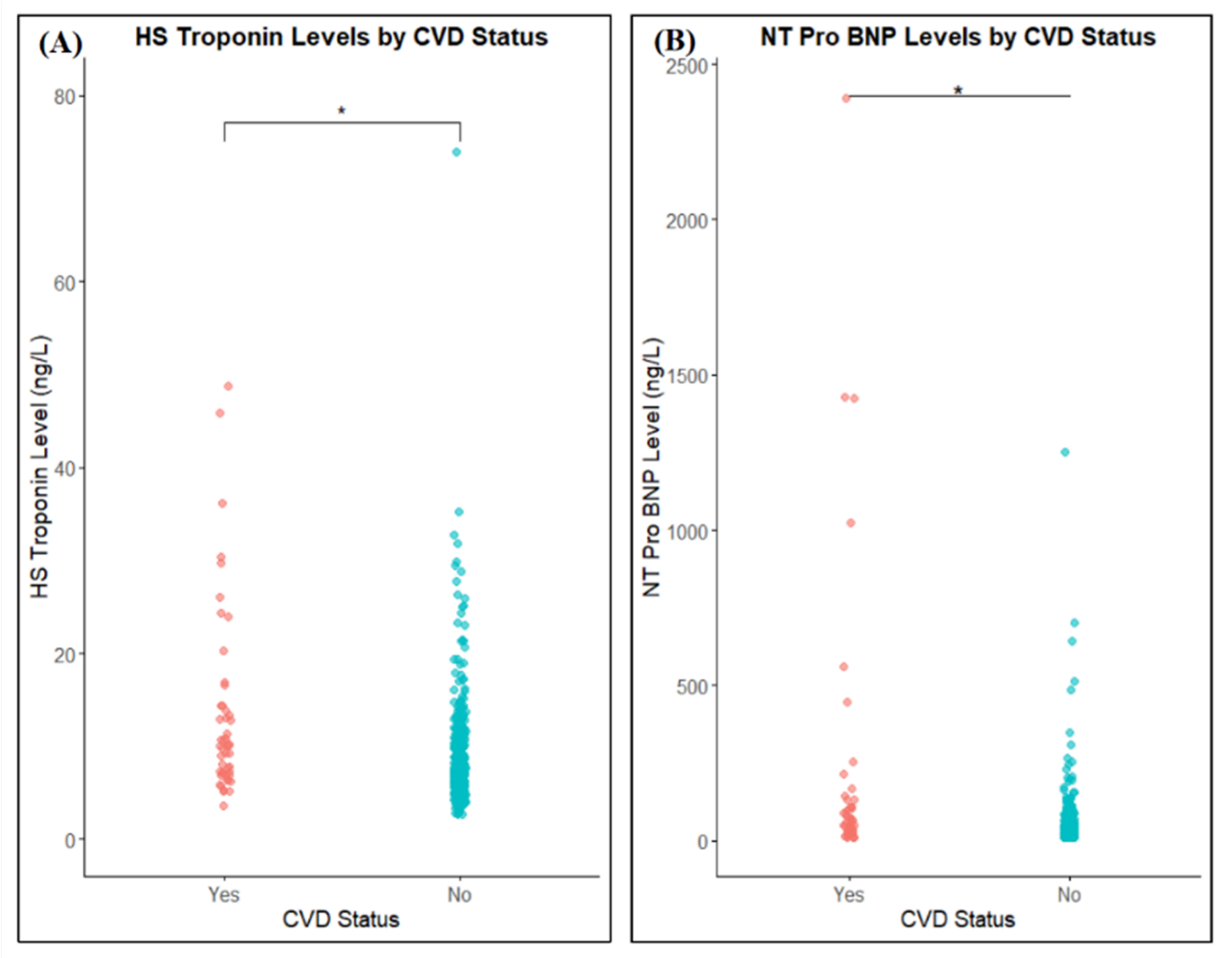


Figure 2

Scatterplots of **(A)** HS-troponin and **(B)** NT-proBNP by CVD status. * Indicates a significant difference at $P < 0.05$

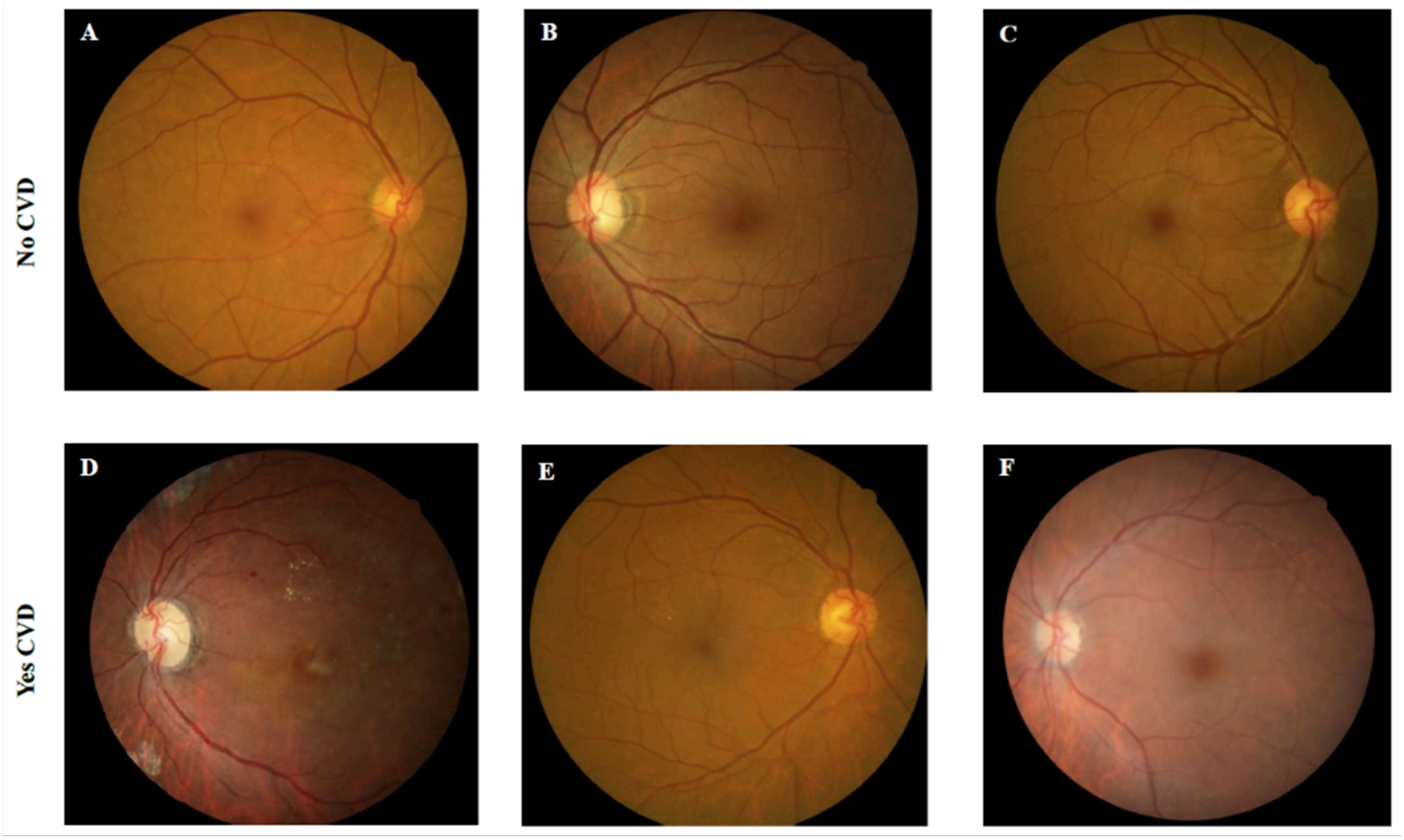


Figure 3

Randomly selected retinal fundus images from participants without cardiovascular disease (CVD) **(A - C)** and with incident CVD **(D - F)**, analysed using the Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE) software version 3.1.0. In these images, venules appear as thicker and darker red, whereas arterioles are thinner and lighted red

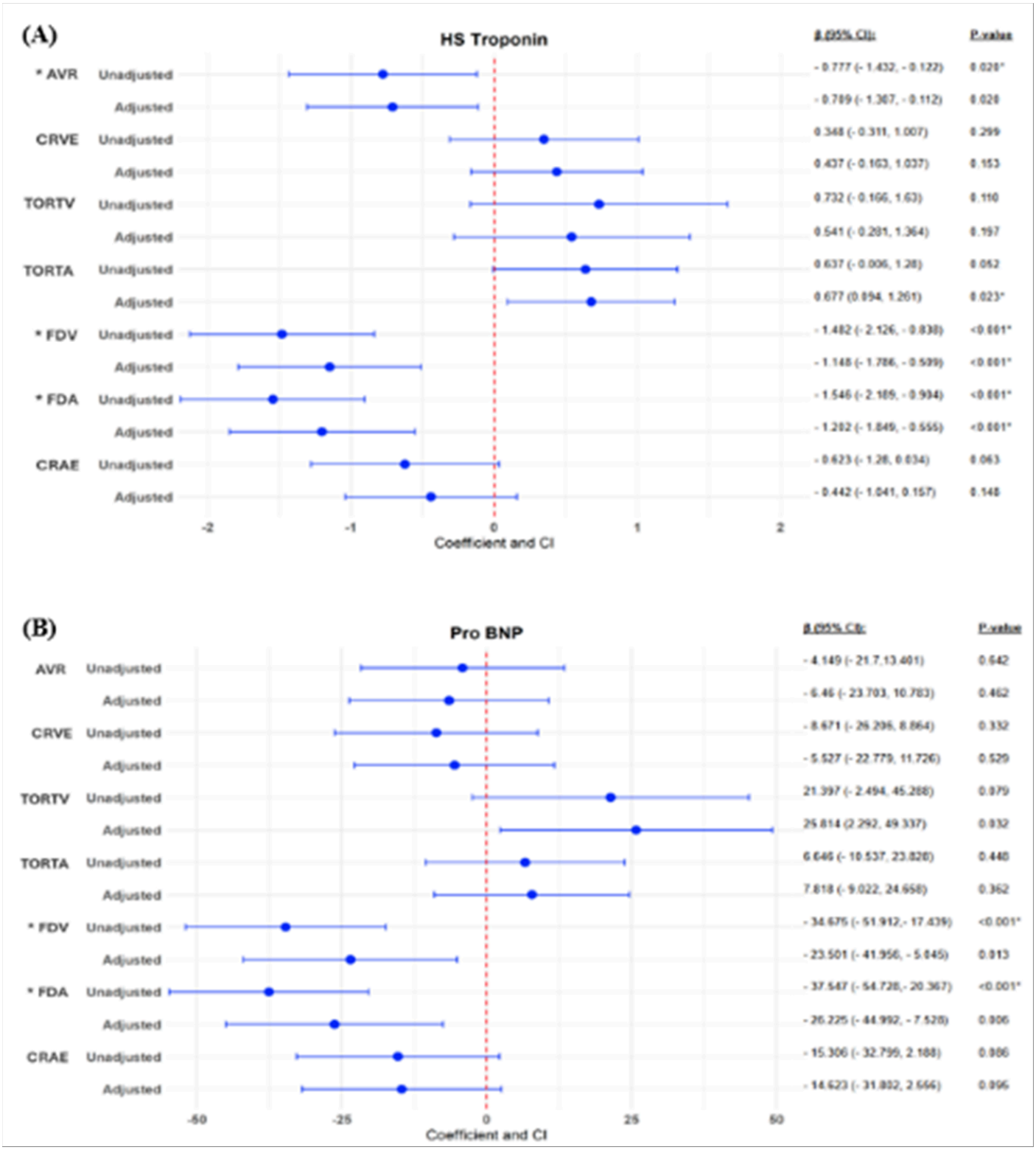


Figure 4

Forest plots for the association between retinal vascular features and cardiac markers: **(A)** HS-troponin and **(B)** NT-proBNP among type 2 diabetes participants in unadjusted β (95% CI): regression coefficient and 95% confidence interval and adjusted models (age and gender).

* Significant $P < 0.05$. Values for arteriole and venule tortuosity were normalized using log e transformation. HS-Troponin: high-sensitivity troponin, NT-proBNP: N-terminal pro b-type natriuretic peptide, CRAE/ CRVE: central retinal arteriole/venule equivalent, TORTA/V: arteriole/venule tortuosity, AVR: arteriovenous ratio, FDA/V: arteriole/venule fractal dimension

Supplementary Files

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