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Original Article

ORIGINAL ARTICLE

Correlation between retinal vascular geometric parameters and pathologically diagnosed type 2 diabetic nephropathy

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ABSTRACT

Background. Diabetic nephropathy (DN) and diabetic retinopathy (DR) are common microvascular complications of diabetes. The purpose of this study was to investigate the correlation between retinal vascular geometric parameters and pathologically diagnosed type 2 DN and to determine the capacity of retinal vascular geometric parameters in differentiating DN from non-diabetic renal disease (NDRD).

Methods. The study participants were adult patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease who underwent a renal biopsy. Univariate and multivariable regression analyses were performed to evaluate associations between retinal vessel geometry parameters and pathologically diagnosed DN. Multivariate binary logistic regression analyses were performed to establish a differential diagnostic model for DN.

Results. In total, 403 patients were examined in this cross-sectional study, including 152 (37.7%) with DN, 157 (39.0%) with NDRD and 94 (23.3%) with DN combined with NDRD. After univariate logistic regression, total vessel fractal dimension, arteriolar fractal dimension and venular fractal dimension were all found to be associated with DN. In multivariate analyses adjusting for age, sex, blood pressure, diabetes, DR and other factors, smaller retinal vascular fractal dimensions were significantly associated with DN ($P < .05$). We developed a differential diagnostic model for DN combining traditional clinical indicators and retinal vascular geometric parameters. The area under the curve of the model established by multivariate logistic regression was 0.930.

Conclusions. Retinal vessel fractal dimension is of great significance for the rapid and non-invasive differentiation of DN. Incorporating retinal vessel fractal dimension into the diagnostic model for DN and NDRD can improve the diagnostic efficiency.

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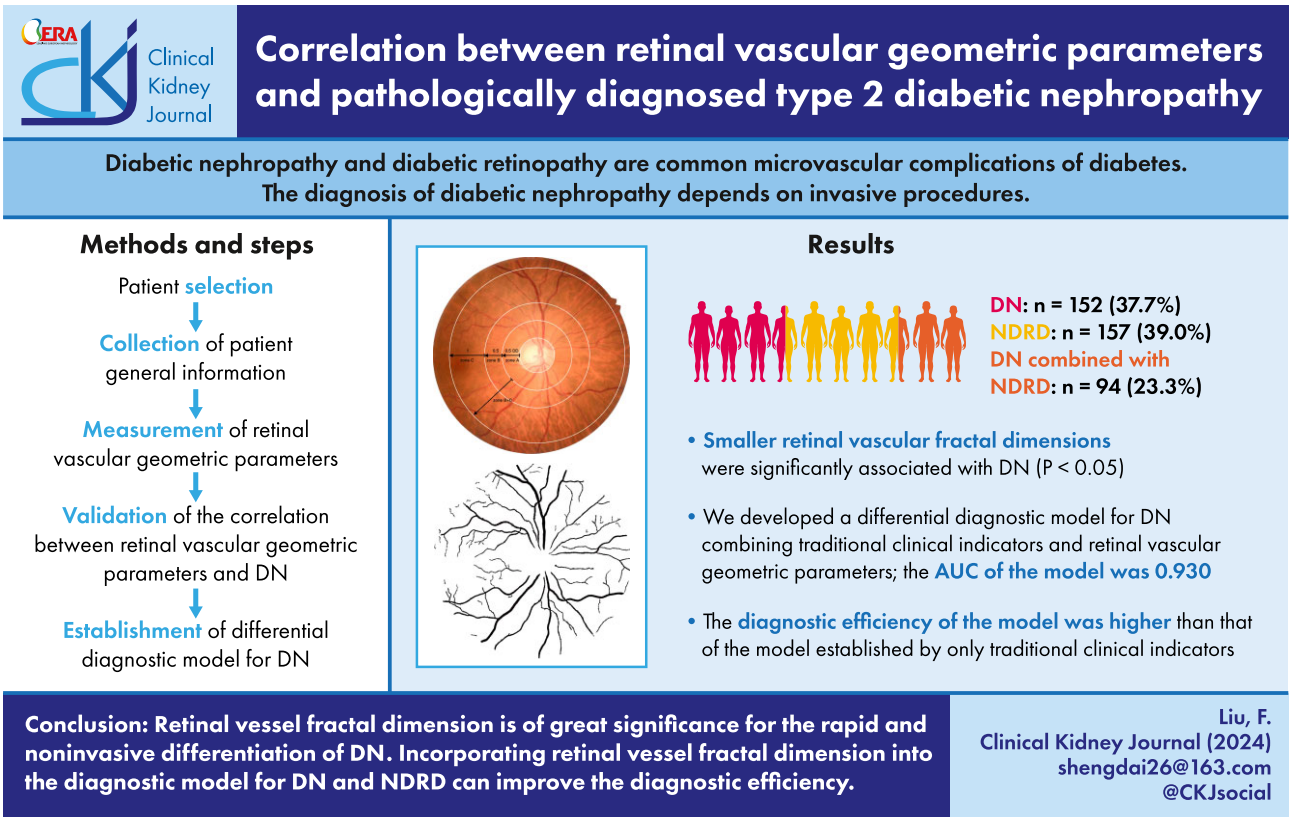
GRAPHICAL ABSTRACT



Keywords: diabetic nephropathy, diabetic retinopathy, non-diabetic renal disease, retinal vascular geometry, type 2 diabetes mellitus

KEY LEARNING POINTS

What was known:

- At present, a large number of studies have shown that the geometric parameters of retinal vessels are significantly related to diabetes and cardiovascular diseases.
- There are no studies on the correlation between retinal vascular geometric parameters and pathologically diagnosed diabetic nephropathy (DN).

This study adds:

- Retinal vessel fractal dimension is of great significance for the rapid and non-invasive differentiation of DN.
- Incorporating retinal vessel fractal dimension into the differential diagnostic model for DN and NDRD can improve the diagnostic efficiency.

Potential impact:

- We analysed the significance of retinal vessel parameters in differentiating DN from NDRD based on renal biopsy results. This will enable early and non-invasive identification of DN, timely determination of treatment plans and delay disease progression.

INTRODUCTION

Diabetes has become a significant global burden, with ≈537 million people 20–79 years of age living with this disease worldwide as of 2021. The International Diabetes Federation predicts that this number will rise to 783 million by 2045 [1]. In China, type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases, affecting ≈140.9 million people [1]. Developing macrovascular (cardiovascular disease) and microvascular [diabetic nephropathy (DN), diabetic retinopathy (DR) and neuropathy] complications during diabetes can lead to blindness, renal failure, a decline in overall quality of life and an increased mortality rate [2]. DN is the most prevalent complication of T2DM

and is the leading cause of end-stage renal disease worldwide [3]. Up to 7% of patients with T2DM are diagnosed with DN [4] and ≈30–40% develop it over time [5]. However, some studies suggest that 33–72.5% of patients with diabetes may have non-diabetic renal disease (NDRD), which can be influenced by the diabetic environment and require different treatment approaches [6]. Diabetic patients with DN have a worse prognosis than those with NDRD, so diagnosis of renal injuries may help stratify them, as accurate diagnosis and differentiation between DN and NDRD are crucial for effective clinical management [7, 8]. Currently, clinicians rely on biomarkers, such as urinary albumin, estimated glomerular filtration rate (eGFR) and haemoglobin levels, as well as patient history and retinopathy status, to differentiate between the two conditions [9–11]. However, these methods are not always reliable. While renal biopsy provides more accurate results [6–8, 12], it is an invasive procedure with potential risks and contraindications, limiting its widespread use in clinical practice [13]. Consequently, simple, rapid and non-invasive methods to distinguish DN and NDRD are needed.

Retinal vessels are a part of the body's microcirculation system and are the only blood vessels in the body that can be directly observed and measured. By examining the retina, a clinician can quickly and non-invasively observe changes in the microcirculation. Research has shown that changes in the geometric parameters of retinal vessels are associated with diabetic complications such as DR and cardiovascular disease [14, 15]. The retinal vessels and glomeruli both belong to the microcirculation system, sharing characteristics of both small blood vessels and organ-specific features. Therefore, when exposed to the same risk factors, they can exhibit similar microcirculation changes [16]. Recent studies have suggested a correlation between retinal vessel parameters and renal damage [17–20]. Consequently, features of the retinal microvascular system can provide basic data on the state of concomitant kidney disease and may predict the risk of kidney disease progression. Renal biopsy is considered the gold standard for distinguishing DN from NDRD [6–8, 21] and is an important basis for epidemiological research on DN, determining clinical–pathological relationships and developing non-invasive diagnostic methods. In this study we analysed the significance of retinal vessel geometry parameters in differentiating DN from NDRD based on renal biopsy results. This will enable early and non-invasive identification of DN, timely determination of treatment plans and delay disease progression.

MATERIALS AND METHODS

Study population

A total of 453 patients with T2DM and chronic kidney disease who underwent a renal biopsy between April 2017 and September 2022 in the Department of Nephrology, First Medical Center of the General Hospital of Chinese PLA were screened. The inclusion criteria were age 18–80 years at renal biopsy (male or female), having biopsy-proven renal lesion and capable of cooperating with fundus photography. The exclusion criteria were having received renal replacement therapy such as dialysis, renal transplantation etc.; incomplete data or an unclear medical history and unclear retinal photographs of both eyes or incomplete parameters for retinal image recognition (Fig. 1). In this study, renal biopsy was performed in patients with renal disease who needed to define the pathological type and develop a treatment plan. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital (no. S2017-133-01). All the participants provided written informed consent.

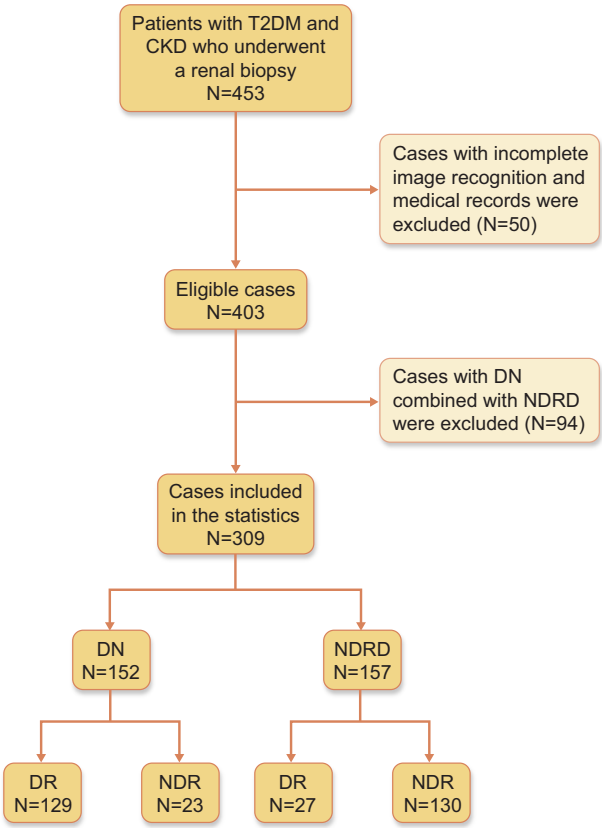


Figure 1: Flow chart illustrating the process of patient screening and the distribution of DR and non-diabetic retinopathy (NDR).

General information and laboratory examination

General patient information, including sex, age, duration of hypertension, duration of diabetes, DR, smoking history, drinking history and blood pressure, was obtained from the related medical records. Body mass index (BMI) was calculated based on height and weight. The examination and test results during hospitalization were obtained from the patients' medical records and included haemoglobin, haemoglobin A1c (HbA1c), serum albumin, urea nitrogen, serum creatinine, blood uric acid, eGFR, cystatin C, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and proteinuria levels. Renal biopsy was performed by experienced doctors and all renal biopsy specimens were independently reviewed by two pathologists and a unified diagnosis was reached after discussion.

Measurement of retinal vascular geometric parameters

We used a VX-20 retinal camera (Kowa, Nagoya, Japan) to capture retinal images of the patient's left and right eyes with a 45° field of view, which included the optic disc and macular area. The retinal image of the right eye is preferred for measurement; if the right eye image could not be captured or the retinal image was not clear, then the left eye image was selected. Cases in which both eye images were unclear were excluded.

Table 1: Data describing the 14 retinal vascular parameters measured for each retinal photograph.

Parameter	Description
Total vessel calibre (CB) Arteriolar calibre (CBa) Venular calibre (CBv) Arteriovenous calibre ratio (AVR)	Median calibre of all identifiable total vessel calibres, arteriolar calibres, venular calibres and ratio of median arteriovenous calibres in zone B.
Total vessel fractal dimension (FD) Arteriolar fractal dimension (FDa) Venular fractal dimension (FDv)	Extract the entire retinal blood vessel tree within the region of the fundus image and calculate the fractal dimension of total vessels, arterioles and venules separately. Fractal dimensions were calculated using a box-counting method [22, 23].
Total vessel tortuosity (TOR) Arteriolar tortuosity (TORa) Venular tortuosity (TORv) Total vessel tortuosity density (TD) Arteriolar tortuosity density (TDa) Venular tortuosity density (TDv)	Calculate the tortuosity of blood vessels in zone C by dividing the arc length by the chord length and use a more complex equation to determine the density of tortuosity [24, 25].
Branching angle (BA)	Calculate the mean value of identifiable branching angles of blood vessels in zone B + C [26].

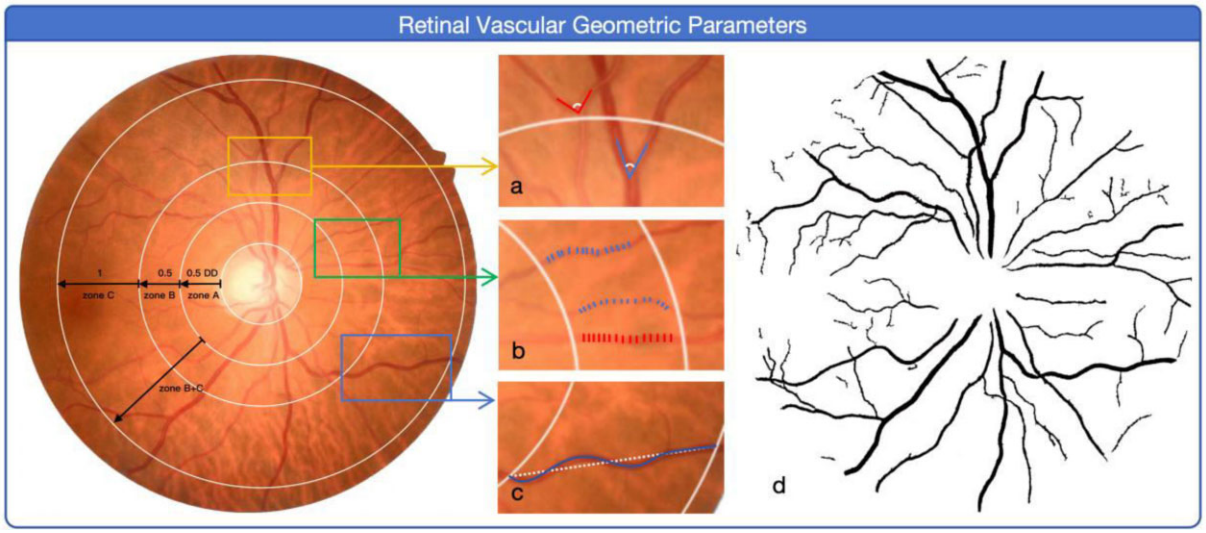


Figure 2: Schematic diagram of **(a)** retinal vessel branching angle, **(b)** retinal vessel calibre, **(c)** retinal vessel tortuosity and **(d)** retinal vessel fractal dimension measurements. Zone A (0–0.5 DD from the edge of the optic disc), zone B (0.5–1 DD from the edge of the optic disc), zone C (1–2 DD from the edge of the optic disc) and zone B + C (0.5–2 DD from the edge of the optic disc).

Retinal vascular geometric parameter measurements were performed using a fully automated computer program written in Python (https://www.python.org/) (Supplementary Fig. S1). The program automatically finds the optic disc centre, segments the whole vascular tree, distinguishes arterioles and venules, measures vessel calibre and calculates fractal dimension, tortuosity and branching angle (Table 1). For more details regarding the computer program, see the supplementary material. The area surrounding the optic disc was divided into three concentric circles, namely zones A, B and C, which are concentric circular areas between 0–0.5 , 0.5–1 and 1–2 disc diameters (DD) away from the edge of the optic disc, respectively. The parameters of specific regions were measured as required (Fig. 2).

Statistical analysis

All data were analysed using SPSS version 26.0 (IBM, Armonk, NY, USA). Continuous data that met the normal distribution were described using mean ± standard deviation (SD), while those that did not meet the normal distribution were described using median [interquartile range (IQR)]. Categorical data were described using composition ratios or percentages. Continuous data were first analysed for normal distribution and homogeneity of variance. Two independent-sample *t*-tests were used to compare two groups of continuous data that met the normal distribution and homogeneity of variance, whereas non-parametric tests were used for those that did not meet these criteria. Retinal vessel parameters were analysed as continuous variables.

Univariate logistic regression analysis was conducted to select variables with $P < .1$. In the multivariate logistic regression analysis, three models were used to analyse the significance of retinal vessel parameters in differentiating between DN and NDRD. To reduce interference, cases of DN combined with NDRD (23.3%) were excluded from this study and only cases of isolated DN and isolated NDRD were included in the statistical analysis. First, to reduce the impact of related factors, age and sex were

Table 2: Baseline characteristics of the study subjects (general information and laboratory results).

Characteristics	Total ($N = 309$)	DN ($n = 152$)	NDRD ($n = 157$)	P-value
General information				
Male, *n* (%)	220 (71.2)	111 (73)	109 (69.4)	.485
Age (years), median (IQR)	54.0 (46.0–59.5)	52.5 (47.0–57.0)	54.0 (46.0–61.0)	.129
Diabetes duration (months), median (IQR)	84 (24–180)	156 (84–204)	36 (10–84)	<.001
Hypertension duration (months), median (IQR)	24 (1–120)	24 (6–120)	24 (0–120)	.127
Smoking, *n* (%)	133 (43)	78 (51.3)	55 (35)	.004
Drinking, *n* (%)	120 (38.8)	64 (42.1)	56 (35.7)	.246
DR, *n* (%)	156 (50.5)	129 (84.9)	27 (17.2)	<.001
BMI (kg/m^2), median (IQR)	26.52 (24.07–29.06)	26.27 (23.71–28.85)	26.72 (24.64–29.07)	0.180
SBP (mmHg), median (IQR)	142.0 (128.0–154.0)	148.0 (133.5–160.0)	136.0 (122.0–148.0)	<.001
DBP (mmHg), median (IQR)	81.0 (74.5–90.0)	82.5 (75.0–90.75)	80.0 (74.0–88.5)	.108
Laboratory results				
Haemoglobin (g/l), mean ± SD	123.04 ± 23.10	115.59 ± 22.23	130.26 ± 21.64	<.001
HbA1c (%), median (IQR)	6.90 (6.10–7.60)	7.10 (6.40–8.00)	6.60 (6.00–6.98)	<.001
Serum albumin (g/l), median (IQR)	34.80 (28.10–39.75)	34.40 (29.25–37.98)	35.20 (27.30–41.10)	.513
Urea nitrogen (mmol/l), median (IQR)	7.71 (5.88–11.38)	9.26 (7.00–13.04)	6.80 (5.15–9.35)	<.001
Serum creatinine (μmol/l), median (IQR)	112.70(82.25–175.90)	140.35(97.00–219.93)	93.30(75.20–134.60)	<.001
Blood uric acid (μmol/l), mean ± SD	366.00 ± 88.84	367.77 ± 82.36	364.22 ± 94.92	.726
eGFR (ml/min/1.73 m^2), median (IQR)	51.19 (30.43–77.00)	39.96 (22.97–62.02)	62.63 (42.24–92.00)	<.001
Cystatin C (mg/l), median (IQR)	1.55 (1.13–2.10)	1.76 (1.41–2.36)	1.29 (1.00–1.76)	<.001
TC (mmol/l), median (IQR)	4.63 (3.74–5.63)	4.36 (3.65–5.29)	4.93 (3.89–6.08)	.004
TG (mmol/l), median (IQR)	2.00 (1.38–2.80)	1.94 (1.28–2.54)	2.06 (1.45–3.07)	.093
HDL-C (mmol/l), median (IQR)	1.01 (0.85–1.19)	0.99 (0.82–1.18)	1.03 (0.86–1.24)	.058
LDL-C (mmol/l), median (IQR)	2.85 (2.10–3.81)	2.71 (1.91–3.60)	2.98 (2.26–4.01)	.006
Proteinuria (g/24 h), median (IQR)	3.18 (1.33–5.59)	4.01 (2.03–6.43)	2.27 (1.02–4.60)	<.001

DBP: diastolic blood pressure.

adjusted in model 1. We further included selected covariates in model 2. Considering the unique correlation between DR and retinal vessel parameters [27], model 3 was developed by incorporating DR into model 2. The effect of collinearity was excluded. P-values <.05 were considered statistically significant and odds ratios (ORs) with 95% confidence intervals (CIs) were provided. If the 95% CI did not cross 1, it was considered statistically significant. Variables were selected based on the results of univariate and multivariate logistic regression statistical analyses and clinical experience and forward stepwise logistic regression was used to select the parameters that ultimately entered the differential diagnostic model.

RESULTS

Baseline characteristics of the population

This study included 403 patients with complete parameter data. Among them, 23.3% were diagnosed with DN combined with NDRD (the Mix group). The baseline characteristics are presented in Supplementary Table S1. To reflect the difference, we excluded patients with DN combined with NDRD and finally included 309 patients in the analysis (Fig. 1). Among the 157 patients with NDRD, membranous nephropathy accounted for the highest proportion (48.4%), followed by immunoglobulin A nephropathy (24.8%).

Baseline characteristics are presented in Tables 2 and 3. No statistically significant differences were observed in age or sex between the DN and NDRD groups. Compared with the NDRD group, the patients in the DN group were more likely to have a long history of diabetes mellitus, smoking history and DR. The DN group had higher levels of systolic blood pressure (SBP), HbA1c, urea nitrogen, serum creatinine, cystatin C and proteinuria and had lower levels of haemoglobin, eGFR, TC, LDL-C and retinal vessel fractal dimension.

Univariate logistic regression analysis

Univariate logistic regression analysis was conducted with DN as the dependent variable. Supplementary Table S2 presents the variables with P-values <.1 in the univariate logistic regression. The results showed that diabetes duration, smoking history, DR, SBP, haemoglobin, HbA1c, urea nitrogen, serum creatinine, eGFR, cystatin C, TC, LDL-C, proteinuria and retinal vessel geometry parameters [total vessel fractal dimension (FD), arteriolar fractal dimension (FDa) and venular fractal dimension (FDv); $P < .001$] were all associated with the diagnosis of DN.

Multivariable logistic regression analysis

Based on our experience and the selected variables ($P < .1$ in the univariate logistic regression), we chose to include age, sex, diabetes duration, smoking history, BMI, SBP, haemoglobin, HbA1c, urea nitrogen, creatinine, eGFR, cystatin C, TC, LDL-C, proteinuria and DR in three models for the stepwise regression analysis after passing the collinearity test. We corrected for these variables to verify whether any differences existed in the retinal vessel geometric parameters between the DN and NDRD groups.

Table 4 presents the results of the multifactor binary logistic regression analysis. After adjusting for multiple variables, FD, FDa and FDv remained statistically significant in differentiating between the DN and NDRD groups ($P < .05$). Figure 3a presents the receiver operating characteristics (ROC) curves for FD, FDa and FDv for independent identification of DN. The areas under the curve (AUC) of the FD, FDa and FDv models were 0.697, 0.617

Table 3: Baseline characteristics of the study subjects (retinal vascular geometric parameters).

Characteristics	Total ($N = 309$)	DN ($n = 152$)	NDRD ($n = 157$)	P-value
CB (pixels)	16.17 (12.58–19.54)	16.35 (12.53–19.54)	16.13 (12.58–19.54)	.907
CBa (pixels)	13.04 (9.18–16.82)	13.15 (9.06–16.71)	12.98 (9.48–16.87)	.830
CBv (pixels)	19.03 (15.26–23.27)	19.47 (15.83–23.66)	19.01 (15.07–23.09)	.623
AVR	0.65 (0.50–0.86)	0.63 (0.49–0.86)	0.66 (0.53–0.86)	.396
FD	1.56 (1.48–1.63)	1.52 (1.42–1.60)	1.59 (1.53–1.65)	<.001
FDa, mean ± SD	1.37 ± 0.13	1.34 ± 0.13	1.40 ± 0.12	<.001
FDv	1.46 (1.37–1.53)	1.41 (1.29–1.50)	1.50 (1.43–1.56)	<.001
TOR	1.031 (1.025–1.039)	1.031 (1.026–1.038)	1.031 (1.024–1.040)	.855
TORa	1.032 (1.023–0.044)	1.033 (1.024–1.040)	1.032 (1.023–1.047)	.445
TORv	1.029 (1.021–1.038)	1.029 (1.022–1.037)	1.028 (1.021–1.038)	.608
TD[a]	4.91 (3.61–6.89)	5.15 (3.62–7.83)	4.65 (3.57–6.45)	.132
TDa[a]	5.20 (3.25–8.73)	5.42 (3.42–9.92)	4.77 (3.08–8.15)	.119
TDv[a]	4.07 (2.69–6.11)	4.08 (2.63–6.34)	4.05 (2.92–5.98)	.856
BA (degrees)	61.71 (51.28–73.76)	62.17 (51.46–72.84)	61.04 (50.65–75.16)	.892

Values are presented as median (IQR) unless stated otherwise.
CB: total vessel calibre; Cba: arteriolar calibre; CBv, venular calibre; AVR: arteriovenous calibre ratio; FD: total vessel fractal dimension; FDa: arteriolar fractal dimension; FDv: venular fractal dimension; TOR: total vessel tortuosity; TORa: arteriolar tortuosity; TORv: venular tortuosity; TD: total vessel tortuosity density; TDa: arteriolar tortuosity density; TDv: venular tortuosity density; BA: branching angle.
[a]For ease of presenting the data, tortuosity densities are calculated here by multiplying the original parameters by four powers of 10.

Table 4: Associations between retinal vascular parameters and DN.

	Model 1[a]		Model 2[b]		Model 3[c]	
Characteristics	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
CB (pixels)	1.00 (0.97–1.01)	.882	1.02 (0.97–1.07)	.547	1.01 (0.95–1.07)	.875
CBa (pixels)	0.99 (0.96–1.03)	.611	1.02 (0.98–1.07)	.336	1.02 (0.96–1.08)	.534
CBv (pixels)	1.00 (0.98–1.03)	.780	1.01 (0.97–1.05)	.573	1.01 (0.96–1.06)	.720
AVR	0.88 (0.48–1.62)	.689	1.31 (0.54–3.12)	.553	1.20 (0.42–3.42)	.732
FD (per 1 SD)	0.38 (0.28–0.51)	<.001	0.40 (0.26–0.60)	<.001	0.39 (0.24–0.65)	<.001
FDa (per 1 SD)	0.57 (0.44–0.73)	<.001	0.61 (0.43–0.87)	.006	0.60 (0.39–0.90)	.015
FDv (per 1 SD)	0.33 (0.24–0.46)	<.001	0.35 (0.23–0.53)	<.001	0.35 (0.21–0.59)	<.001
TOR (per 1 SD)	0.95 (0.76–1.19)	.648	0.87 (0.63–1.21)	.411	0.86 (0.60–1.25)	.441
TORa (per 1 SD)	0.89 (0.71–1.12)	.303	0.75 (0.54–1.03)	.075	0.69 (0.48–1.00)	.051
TORv (per 1 SD)	1.06 (0.85–1.33)	.616	1.16 (0.84–1.59)	.365	1.18 (0.81–1.72)	.394
TD (per 1 SD)	1.09 (0.87–1.37)	.464	1.14 (0.86–1.51)	.380	1.10 (0.79–1.52)	.576
TDa (per 1 SD)	1.20 (0.95–1.52)	.120	1.15 (0.84–1.59)	.382	1.10 (0.74–1.55)	.712
TDv (per 1 SD)	0.91 (0.72–1.14)	.406	1.02 (0.76–1.37)	.900	1.00 (0.70–1.43)	.995
BA (degrees)	1.00 (0.99–1.01)	.967	1.00 (0.98–1.01)	.575	1.00 (0.98–1.01)	.620

Multivariate binary logistic regression analysis. Dependent variable: DN. Fractal dimension, tortuosity and tortuosity density were treated as continuous per increment in 1 SD.
[a]Model 1 adjusted for age and sex.
[b]Model 2 adjusted for age, sex, duration of diabetes, smoking, BMI, SBP, haemoglobin, HbA1c, urea nitrogen, serum creatinine, eGFR, cystatin C, TC, LDL-C and proteinuria.
[c]Model 3 adjusted for age, sex, duration of diabetes, smoking, BMI, SBP, haemoglobin, HbA1c, urea nitrogen, serum creatinine, eGFR, cystatin C, TC, LDL-C, proteinuria and DR.

and 0.719, respectively. Retinal vessel fractal dimension could be a potential clinical biomarker for DN. FDv was the parameter with the highest diagnostic efficiency among the three fractal dimensions.

Establishment of the differential diagnosis model

We first selected the traditional clinical indicators associated with DN based on the results of univariate logistic regression and clinical experience and that passed the test of collinearity. The independent variable was screened by the forward stepwise logistic regression method. We identified the duration of diabetes, DR, LDL-C, SBP and HbA1c as the five parameters in the DN model ($P < .001$, $P < .001$, $P = .002$, $P = .007$ and $P = .075$, respectively). The AUC of the model was 0.923 (95% CI 0.891–0.954, $P < .001$).

We then combined traditional clinical indicators and retinal vascular geometric parameters, screened the variable in the same way and determined if it passed the test for collinearity (for variables of retinal vessel fractal dimension with high collinearity, the FDv with the highest diagnostic efficacy was chosen to enter the model). By forward stepwise multivariate logistic regression analysis, we identified the duration of diabetes (Dm), DR, FDv, LDL-C and SBP as independent correlating factors of DN ($P < .001$, $P < .001$, $P < .001$, $P = .001$ and $P = .019$, respectively) (Table 5).

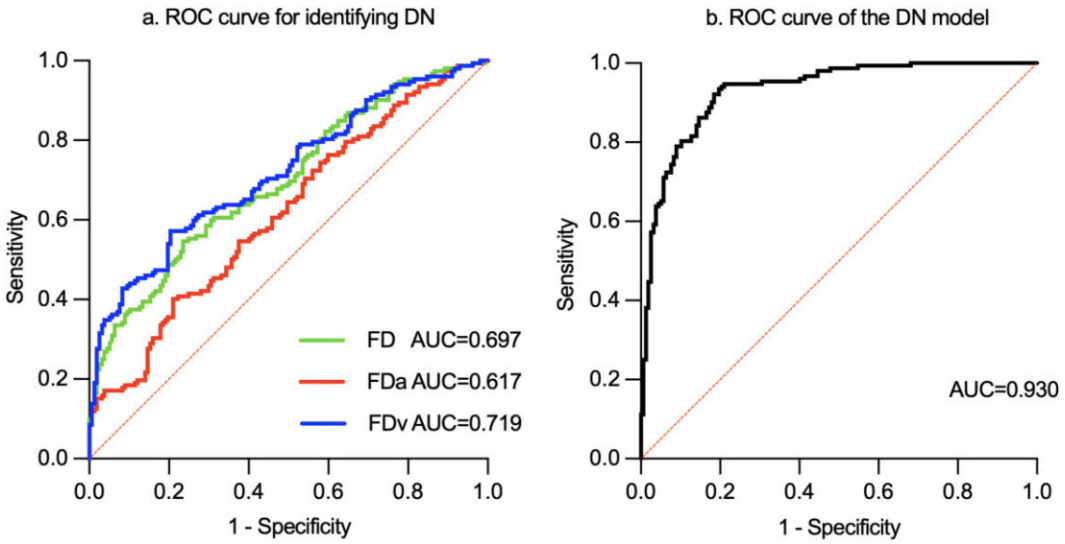


Figure 3: ROC curves for identifying DN. **(a)** The ROC curve of FD, FDa and FDv for independent identification of DN. The AUCs were 0.697, 0.617 and 0.719, respectively. **(b)** The ROC curve of the DN model established by traditional factors and retinal vascular geometric parameters. The AUC was 0.930, with a 95% CI of 0.903–0.958 and a P-value <.001.

Table 5: Multivariate regression analysis results.

Variables	β	SE	P-value	OR
Traditional factors model				
Diabetes duration (months)	0.012	0.002	<.001	1.012
DR (1: yes; 0: no)	2.928	0.363	<.001	18.698
LDL-C (mmol/l)	−0.46	0.147	.002	0.631
SBP (mmHg)	0.023	0.008	.007	1.023
HbA1c (%)	0.238	0.134	.075	1.268
Traditional factors and retinal vascular parameters model				
Diabetes duration (months)	0.012	0.002	<.001	1.012
DR (1: yes; 0: no)	2.812	0.371	<.001	16.643
FDv	−5.536	1.654	<.001	0.004
LDL-C (mmol/l)	−0.462	0.145	.001	0.63
SBP (mmHg)	0.02	0.009	.019	1.021

Dependent variable: DN.
β: coefficient value; SE: standard error.

The DN diagnostic model was constructed as follows:

$$P_{DN} = \exp\left(3.840 + 0.012\text{Dm} + 2.812\text{DR} - 5.536\text{FDv} - 0.462\text{LDL-C} + 0.020\text{SBP}\right)/[1 + \exp(3.840 + 0.012\text{Dm} + 2.812\text{DR} - 5.536\text{FDv} - 0.462\text{LDL-C} + 0.020\text{SBP})]$$

P_{DN} represents the probability of a DN diagnosis ($P_{DN} \geq .5$ as DN, $P_{DN} < .5$ as NDRD). Based on the binary logistic regression equation, we plotted ROC curves. The AUC was 0.930 (95% CI 0.903–0.958, $P < .001$; Fig. 3b). The results show that incorporating retinal vascular fractal dimension into the differential diagnostic model for DN and NDRD can improve the diagnostic efficiency.

As a side note, we attempted to include the DN + NDRD group (Mix group) in the DN and NDRD groups separately, similarly using forward stepwise logistic regression to screen for the five variables most strongly associated with DN (including FDv), and found that the differential diagnostic equations established all had an AUC of 0.871. The inclusion of the Mix group may affect the accuracy of our results, it is often the case that renal puncture is considered in patients with relatively complex or poorly judged conditions and therefore the proportion of renal puncture patients in the Mix group is not a true proportion of the diseased population. After consideration, we excluded patients with DN combined with NDRD to build the model. We applied the DN model to 403 patients with complete parameters (including 94 cases in the Mix group) in this study. The AUC of the model for identifying DN(± NDRD) was 0.861 and the AUC for identifying isolated DN was 0.870, which still had high accuracy (Supplementary Fig. S2).

DISCUSSION

DN is characterized by severe systemic metabolic disorders. Diabetes can not only directly progress to DN but can also affect the progression of NDRD [6]. Therefore, timely identification of DN versus NDRD and early intervention is essential.

DN and DR are the most common microvascular complications of T2DM. Studies have shown that the same regulatory factors are involved in both DN and DR [28]. Retinal vessel fractal dimension is closely related to the occurrence and progression of DR [29] and significantly correlates with the renal function indicators (eGFR, urinary microalbumin, albumin:creatinine ratio (ACR), creatinine, albumin and cystatin C) [17, 30]. Low retinal vascular fractal dimension was significantly associated with higher risks for incident mortality, hypertension, renal failure, T2DM, anaemia and multiple ocular conditions [31]. Considering these relevant factors, this study used three stepwise regression models. After adjusting for age, sex, duration of diabetes, smoking, BMI, SBP, haemoglobin, HbA1c, urea nitrogen, serum creatinine, eGFR, cystatin C, TC, LDL-C, proteinuria and DR in model 3, the differences in fractal dimension (FD, FDa and FDv) between the two groups remained statistically significant. As retinal vessel fractal dimension is a continuous variable, we speculated that changes in retinal vessel fractal dimension may precede

the onset of DR. Compared with other traditional clinical indicators, the retinal vascular geometric parameters do not need to be diagnosed by doctors and do not need invasive surgery. In summary, fractal dimension is an effective parameter for distinguishing between DN and NDRD. After multivariate correction, the differences of FD, FDa and FDv between the two groups showed statistical significance.

Fractal dimension reflects the effectiveness of the space occupied by a complex form and is a measure of the irregularity of the complex form. The retinal vascular fractal dimension reflects the complexity and density of the vasculature, with larger values reflecting greater structural complexity. Abnormal alterations in FDv attributed to dysregulation of vascular growth, either by abnormal vascular proliferation or insufficient angiogenesis, can cause disease [32, 33]. Rarefaction of the retinal vasculature is thought to reflect suboptimal retinal vascular branching complexity, which may reflect poor ocular blood flow in disease [33, 34]. Although DR tends to lead to retinal vascular proliferation, it does not change the eventual deterioration of the vascular network and the reduction in vascular fractal dimension. The extent of proliferation may differ between arteries and veins, which could explain the variation in their diagnostic efficacy.

Even if other studies have shown retinal vessel fractal dimension correlation with the progression of DR and renal function, patients with both DR and renal impairment do not necessarily have DN. After we corrected those indicators, the fractal dimension of retinal blood vessels still had a strong correlation with pathologically diagnosed DN. The occurrence of DN is a complex process. In addition to the direct effect of diabetes, other common diseases, such as cardiovascular disease, are also closely related to it. Blockade of the renin–angiotensin system exerts a protective role in DN regardless of the presence of arterial hypertension, although it has a less effective role in the treatment of DR [35]. Moreover, recently, for the first time in a randomized controlled trial, the Nephropathy in Type 2 Diabetes and Cardiorenal Events study (NCT00535925), the association between DN and DR was shown to identify subjects at very high cardiovascular risk, even if on primary cardiovascular prevention, and that an intensive multifactorial pharmacotherapy treatment can reduce global mortality and major adverse cardiac events [36]. Therefore, the treatment of DN needs comprehensive consideration.

Previous studies have shown that retinal vascular geometric parameters are associated with renal damage and diabetes [17–20, 37]. A lower retinal vascular fractal dimension was consistently associated with the 16-year incidence of DN in a prospective study of young Danish patients with type 1 diabetes, but participants with macroalbuminuria (ACR $\geq$300 mg/g), with a history of kidney transplantation or who had received dialysis were all classified as having DN in this study. It was demonstrated that retinal vascular fractal dimension correlates with biomarkers and events of renal damage [38]. However, no study has explored the significance of retinal vascular geometric parameters in differentiating DN from NDRD. Our study has the following advantages. First, renal biopsy is currently the gold standard for differentiating DN from NDRD, our study relied on the pathological reports from renal biopsies to avoid misdiagnosis caused by relying solely on clinical characteristics. Accordingly, highly accurate models were established. Second, we used machine learning methods to identify and distinguish arteriovenous blood vessels and an independently developed computer program to measure retinal vascular parameters, improving the accuracy of the parameters. Third, many previous studies have analysed retinal vascular parameters using software to differentiate between arteries and veins [17–20]. We classified different parameters and included both total vascular parameters and arteriovenous vascular parameters for analysis to differentiate DN.

However, our study has some limitations. First, all patients included in this study underwent a renal biopsy and had a clear pathological diagnosis. However, owing to certain contraindications, invasive procedures and limited patient acceptance, renal biopsy was not performed in all patients with T2DM and renal impairment, thus we cannot know the true prevalence of DN and NDRD in patients with T2DM [12]. Second, the measurement methods of fundus parameters are diverse and different measurement methods may obtain different results, which needs further comparison and research. Third, this study had a limited sample size, was a single-centre study and lacked long-term follow-up data, thus the correlation between changes in retinal vascular geometric parameters and progression of nephropathy in patients was not obtained. Despite these limitations, this study provides novel ideas and methods.

In conclusion, we found that retinal vessel fractal dimension is of great significance for the rapid and non-invasive differentiation of DN from NDRD; the lower the retinal vessel fractal dimension, the more likely the patient is to have DN, and retinal vessel fractal dimension is a potential biomarker for DN. The differential diagnostic model of DN and NDRD established by retinal vessel fractal dimension and clinical indicators has a high diagnostic efficiency and provides quantitative indicators for clinical judgment to improve the scientific nature of disease diagnosis and reduce medical risks.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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