Measurement of Macular Fractal Dimension Using a Computer-Assisted Program

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Purpose. Macular diseases may be associated with an altered retinal vasculature. We describe and test new software for the measurement of retinal vascular fractal dimension to quantify the complexity of retinal vasculature at the macula (D_{mac}) and to compare this with fractal dimension measured around the optic disc (D_{disc}).

Methods. A total of 342 macular-centered and optic disc-centered digital retinal photographs from 171 subjects was selected randomly from a population-based study. Retinal vascular fractional dimension (D_f) was measured by two trained graders using a computer-assisted program (SIVA-FA, software version 1.0, National University of Singapore) on macula-centered (D_{mac}) and optic disc-centered (D_{disc}) photographs, to assess intergrader reliability. Measurements were repeated after two weeks to determine intragrader reliability. A separate 50 pairs of consecutively repeated images were selected and measured using SIVA-FA to assess intrasession reliability. Reliability analyses were conducted using intraclass correlation coefficients (ICC), and multiple linear regression analyses were performed to compare factors associated with D_{mac} and D_{disc} measurements.

Results. The mean (SD) D_{mac} and D_{disc} values were 1.453 (0.060) and 1.484 (0.043), respectively, and were highly correlated ($r = 0.70$, $P < 0.001$). Intragrader, intergrader, and intrasession reliability for both D_f measures was high (ICCs ranging from 0.88–0.99). In multiple regression analyses, age (both $\beta = -0.03$, $P < 0.001$) and hypertension ($\beta = -0.02$, $P = 0.011$; $\beta = -0.02$, $P = 0.021$, respectively) were independently associated with D_{mac} and D_{disc}.

Conclusions. The complexity of the retinal vasculature in the macula can be measured reliably and may be a useful tool to study parafoveal vascular networks in macula diseases, such as diabetic maculopathy.

Keywords: macula, fractal, retinal vasculature

The major contributor to visual acuity and central vision is the macula and, hence, its associated diseases often cause significant visual impairment and blindness.[1] Major causes of macular disease, including diabetic macular edema and age-related macular degeneration, often are detected only once significant and disabling visual loss has occurred, hence the need for earlier diagnosis. In diabetic retinopathy and maculopathy, there are established changes[2,3] to the retinal vasculature during the subclinical phases of disease, which provide an opportunity for early noninvasive diagnosis and, therefore, intervention to prevent disease progression.

In healthy participants, physiological systems are designed for maximal efficiency to reduce the work expended by the body. In disease states, there often are structural changes (e.g., to blood vessel walls) that may change the configuration of these physiological systems so it performs less efficiently, for example, the remodeling of blood vessels causing increased tortuosity or altered branching patterns.[4] Changes in the retinal vascular architecture may reflect impaired microcirculatory transport, nonuniform shear distribution across bifurcations and reduced energy efficiency in blood flow.[4] These pathological changes, even in the absence of clinical symptoms, can now be detected via methods that analyze the structure of the retinal vessels, and may be reflective of vascular disease.[4] The fractal dimension (D_f) of the retinal vasculature reflects the geometry and complexity of retinal vessel branching architecture.[5–10] Previous work has employed computerized methods to measure the D_f centered principally on the optic disc (D_{disc}).[11] Based on this method, studies have reported associations of D_{disc} with a range of retinal vascular diseases, including hypertensive and diabetic retinopathy,[12–14] and

systemic disorders, such as hypertension[11,15] and diabetes mellitus.[12,16–20]

However, diseases of the macula may be related more closely to changes in blood vessels around the macula.[2,3,21,22] To date, there are limited studies that describe the measurement D_f of retinal vessels at the macula specifically, using automated software that is suitable for use in the clinic. To address this gap, we developed a computer-assisted software to measure the D_f of macula-centered photographs (D_{mac}) and compared this with D_f of optic disc-centered photographs (D_{disc}). We described the reliability of these D_f measurements and compared associations with systemic and ocular factors.

Methods

Study Population

Retinal photographs from the Singapore Indian Eye Study (SINDI), a cross-sectional population-based survey of 3400 persons aged more than 40 years, were used for this study. The objectives and methodology of the SINDI Population Based Study, of which this is a substudy, has been reported in detailed previously.[23] This study adhered to the tenets of the Declaration of Helsinki and ethics committee approval was obtained from the Institutional Review Board of the Singapore Eye Research Institute (SERI). Written informed consent was obtained from all participants. A random 5% of the total SINDI population ($n = 171$) was chosen manually for the analysis via case number by a human operator without any patient identifiers.

Retinal Photography

Two 45° digital retinal photographs of macula and optic disc-centered fields were obtained from each participant's eye after pupil dilation. All photographs were taken using a digital retinal camera (Canon CR-DGi with a Canon 10D SLR body; Canon, Tokyo, Japan), following a standardized protocol (i.e., flash settings, brightness, contrast, exposure times, and aperture). In this study, we used the optic disc-centered and macular-centered photographs of the right eye of each participant; if the right eye photographs were ungradable, the measurement was performed on the left eye.

Measurement of Fractal Dimension of Retinal Photographs

We developed a new semiautomated software package, Singapore I Vessel Assessment-FractalAnalyzer (SIVA-FA software version 1, National University of Singapore, Singapore), to measure the D_f of digital fundus images. Trained graders masked to participants' characteristics performed the fractal measurement according to a standardized protocol. In brief, the image type first was selected (optic disc-centered or macula-centered field) and optic discs were detected automatically by the detection of the edges of the optic nerve head by the software. The region of interest (the measured area) was defined relative to the position and size of the optic disc for each individual. The overall effect of optic disc size on D_{mac} and D_{disc} was expected to be minimal, as theoretically the fractal dimension calculates the complexity and branching patterns of a fractal structure irrespective to the area of analysis. This was followed by automated skeletonized tracing of the retinal vessels generated by the software. To further ascertain the accuracy of the automated skeletonized vessel tracing, the graders examined the skeletonized vessel tracing and compared it to the original image to identify and erase artifacts that occasionally were included erroneously in the skeletonized vessel tracing, such as peripapillary atrophy, choroidal vessels, and pigment abnormalities. After vessel tracing was ascertained, the program then computed D_f from the refined skeletonized vessel tracing using the Box-Counting method. The box counting equation, briefly is:

$$D_f = \lim_{r \to 0} \log(N[r]) / \log(1/r)$$

where $N(r)$ is the number of boxes overlying a fractal structure and r is the side length of each box.

Briefly the box counting method involves drawing boxes of side length "r" over a given fractal structure and the number of boxes overlying the structure is $N(r)$. This is repeated for many side lengths and the fractal dimension is related to how the ratio of the box area "$N(r)$" scales with "r," as r approaches the limit of 0. This method is an established technique used to measure the D_f of real-life structures, and the derivation and application of this formula has been described in detail previously.[6,10,11] The overall grading time of an image was approximately 2 minutes. The D_{mac} was measured from macula-centered images. The measured area of D_{mac} was defined as the region with diameter 5 times the optic disc diameter, with its medial border displaced 1/4 of a disc diameter nasally to the nasal border of the optic disc (Fig. 1A). This displacement allows optimal inclusion of the major retinal vessels for macula-centered images. The D_{disc} was measured from optic disc-centered images. The measured area of D_{disc} was defined as the region from 0.5 to 2.0 optic disc diameters away from the disc margin (Fig. 1B). The calculation of D_{disc} and cropping of its measured area were performed using the same method as with an existing D_{disc} measurement computer-assisted program.[24]

Reliability

Two trained graders (S-YO and JA-C) measured the D_{disc} and D_{mac} from digital fundus photographs independently to determine intergrader reliability. The graders repeated the measurements after two weeks to determine intragrader reliability. In addition, intrasession reliability was assessed by one trained grader, measuring D_{disc} and D_{mac} for 50 pairs of repeatedly taken optic disc- and macula-centered images, respectively.

Statistical Analysis

Statistical analyses were conducted using the SPSS Statistics software, version 17.0 (SPSS, Inc., Chicago, IL, USA). Intergrader, intragrader, and intrasession reliability was assessed using intraclass correlation coefficients (ICC). With the given sample size ($n = 171$) and number of raters ($n = 2$), we had a statistical power to provide a 95% confidence interval (CI) at approximately ICC = 0.85 with width 0.2 inches.

Independent t-tests, Pearson's correlation analyses, and multiple linear regression analyses were performed to examine the effects of a range of ocular (e.g., IOP, axial length, spherical equivalent, central corneal thickness, presence of cataract) and systemic (e.g., blood pressure, serum glucose, cholesterol) factors on D_{disc} and D_{mac}. In multiple linear regression analyses, only factors that were significant in univariate analyses ($P < 0.05$) or of scientific importance were included in the model.

Results

Table 1 shows the characteristics of the participants in this study. Of the 171 participants in this study, 50% were male. The

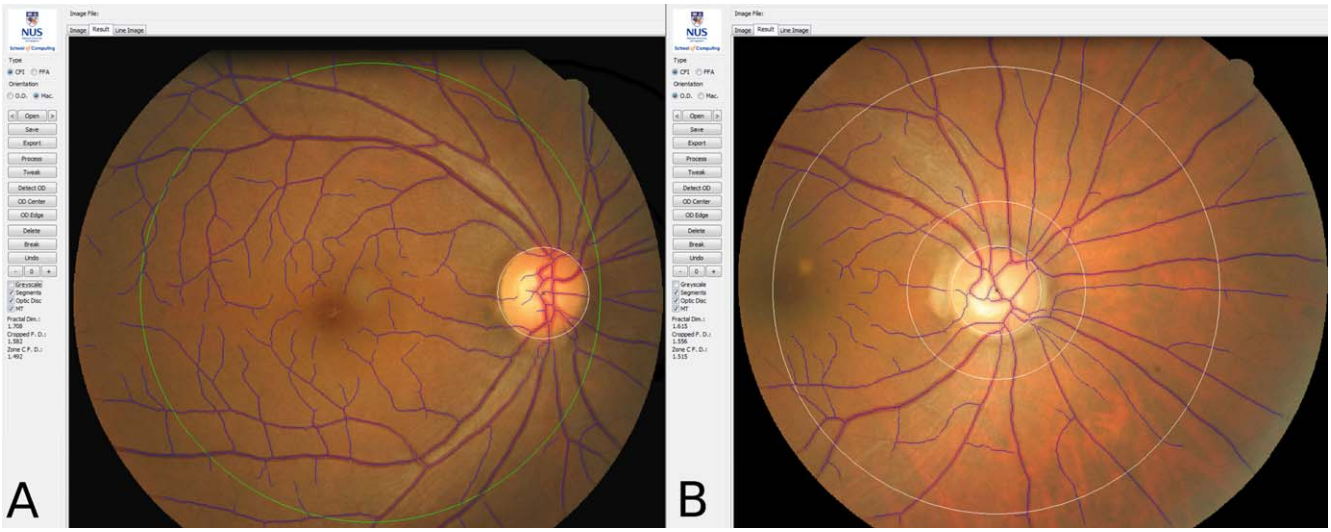

Figure 1. Fractal analysis for color retinal fundus photographs using SIVA-FA. (**A**) Macula-centered image. (**B**) Optic disc-centered image.

mean (SD) age was 56 (9.39) years. The mean systolic (SBP) and diastolic (DBP) blood pressures were 135 (18.4) and 79 (9.65) mm Hg, respectively. The mean D_{disc} and D_{mac} (SD) were 1.484 (0.043) and 1.453 (0.060), respectively. The D_{disc} was significantly larger than D_{mac} ($P < 0.001$). In addition, D_{disc} and D_{mac} were highly correlated ($r = 0.701$, $P < 0.001$).

Table 2 shows the intragrader, intergrader, and intrasession reliability estimates for D_f, with ICCs ranging from 0.88 to 0.99.

Table 3 shows the univariate analyses between D_{mac}, D_{disc}, and various systemic and ocular factors. The D_{mac} was correlated more strongly than D_{disc} with age ($r = -0.48$ and -0.44, respectively, all $P < 0.001$), SBP ($r = -0.25$ and -0.22, respectively, all $P \leq 0.004$), and pulse pressure ($r = -0.28$ and -0.23, respectively, all $P \leq 0.002$). Hypertensive subjects had significantly lower D_{mac} and D_{disc} than healthy subjects ($P < 0.001$). In the univariate analyses of D_{mac} and D_{disc} with continuous factors, only spherical equivalent was correlated significantly with D_{mac} and D_{disc} ($r = -0.19$, $P = 0.017$ and $r = -0.18$, $P = 0.024$, respectively). In the univariate analyses D_{mac} and D_{disc} with categorical factors, cataract was correlated significantly with reduced D_{mac} (1.498 vs. 1.543, $P = 0.003$) and D_{disc} (1.355 vs. 1.393, $P < 0.001$).

Table 4 shows the multiple linear regression analyses of D_{mac} and D_{disc} with systemic and ocular risk factors. Age was associated independently with D_{mac} and D_{disc} (both $\beta = -0.03$, $P < 0.001$). Presence of hypertension also was associated independently with D_{mac} and D_{disc} (both $\beta = -0.02$, $P = 0.012$ and 0.021, respectively). However, the associations between D_{mac} and D_{disc} with sex, spherical equivalent, and presence of cataract were attenuated in the multivariate models. Age had the strongest effect on D_{mac} and D_{disc} measurements ($s\beta = -0.41$ and $s\beta = -0.43$, respectively).

Table 1. Characteristics of Participants

Characteristics	Mean	SD	*n* (%)
Age, y	55.8	9.4	
SBP, mm Hg	135.0	18.4	
DBP, mm Hg	78.7	9.7	
Body mass index, kg/m^2	26.5	4.8	
Total cholesterol, mmol/L	5.2	1.1	
HDL cholesterol, mmol/L	1.06	0.27	
LDL cholesterol, mmol/L	3.43	0.89	
Blood glucose, mmol/L	7.58	3.93	
HbA1c, %	6.69	1.51	
Axial length, mm	23.5	1.1	
Anterior chamber depth, mm	3.25	0.36	
Central corneal thickness, μm	543.4	35.0	
Corneal curvature, mm	7.61	0.25	
Spherical equivalent, diopter	−0.08	1.89	
IOP, mm Hg	15.5	2.6	
Sex, male			86 (50.3)
Diabetes, yes			59 (36.0)
Hypertension, yes			97 (57.1)
Age-related macular degeneration, yes			11 (6.5)
Retinopathy, yes			35 (20.7)
Cataract, yes			45 (26.3)

HbA1c, hemoglobin A1c.

Discussion

Macular retinal vascular D_f is a structural descriptor of the vasculature around the macula, and may be a potential marker for vascular-related macular and systemic diseases. In this study, we showed that the newly developed SIVA-FA has excellent reliability in D_{mac} and D_{disc} measurements. To the best of our knowledge, this new software package is the first self-contained program to allow reliable, automated segmentation and skeletonization of the retinal vessels as well as quantitative vascular fractal measurement at the macular

Table 2. Intragrader, Intergrader, and Intrasession Reliability of Retinal Vascular Fractal Dimension Measurement Using the SIVA-FA Software

	ICC (95% CI)	
	D_{mac}	D_{disc}
Intragrader		
Graders 1 vs. 1	0.99 (0.98–0.99)	0.95 (0.93–0.96)
Graders 2 vs. 2	0.97 (0.97–0.98)	0.97 (0.96–0.98)
Intergrader		
Graders 1 vs. 2	0.88 (0.85–0.91)	0.93 (0.91–0.95)
Intrasession		
Shots 1 vs. 2	0.93 (0.89–0.96)	0.99 (0.976–0.99)

Table 3. Relationship of Retinal Vascular Fractal Dimension With Systemic and Ocular Factors

	D_{mac}			D_{disc}		
	r	Mean (SD)	*P*	*r*	Mean (SD)	*P*
Continuous systemic factors						
Age, y	−0.48		<0.001	−0.44		<0.001
SBP, mm Hg	−0.25		0.001	−0.22		0.004
DBP, mm Hg	−0.03		0.715	−0.05		0.525
Pulse pressure, mm Hg	−0.28		<0.001	−0.23		0.002
Body mass index, kg/m^2	0.03		0.680	−0.02		0.825
Blood glucose, mmol/L	0.00		0.979	0.06		0.452
Creatinine, mmol/L	0.01		0.882	0.05		0.552
Total cholesterol, mmol/L	0.04		0.623	0.02		0.813
HDL cholesterol, mmol/L	−0.04		0.601	−0.01		0.923
LDL cholesterol, mmol/L	0.08		0.327	0.05		0.572
Continuous ocular factors						
Axial length, mm	0.11		0.159	0.07		0.349
Anterior chamber depth, mm	0.13		0.097	0.14		0.068
Central corneal thickness, mm	0.10		0.181	0.13		0.081
Corneal curvature, mm	0.05		0.493	0.06		0.424
Spherical equivalent, diopter	−0.19		0.017	−0.18		0.024
IOP, mm Hg	0.04		0.652	0.02		0.815
Categorical systemic and ocular factors						
Sex						
Male		1.530 (0.060)	0.769		1.387 (0.062)	0.309
Female		1.528 (0.059)			1.277 (0.061)	
Smoking status						
Current		1.538 (0.062)	0.409		1.408 (0.043)	0.020
Past/never		1.527 (0.059)			1.378 (0.063)	
Hypertension						
Yes		1.511 (0.062)	<0.001		1.366 (0.064)	<0.001
No		1.552 (0.047)			1.403 (0.052)	
Cataract						
Yes		1.498 (0.070)	<0.001		1.355 (0.076)	0.003
No		1.543 (0.049)			1.393 (0.051)	
Age-related macular degeneration						
Yes		1.515 (0.063)	0.406		1.366 (0.060)	0.354
No		1.530 (0.059)			1.384 (0.061)	
Retinopathy						
Yes		1.512 (0.071)	0.122		1.369 (0.079)	0.280
No		1.534 (0.056)			1.386 (0.057)	

HDL, high-density lipoprotein; LDL, low-density lipoprotein; *r*, Pearson correlation coefficients.

region. This new feature may provide an opportunity to evaluate the parafoveal vascular network, which may help us to gain insight into the pathogenesis of vascular-related macular diseases, such as diabetic macular edema and age-related macular degeneration. In the multivariate regression analysis, we found that older age and hypertension are associated significantly with lower D_{mac} and D_{disc}, with D_{mac} being a stronger predictor.

We demonstrated high intragrader, intergrader and intra-session reliability using this new program. Similarly, previous studies that measure D_f from optic disc-centered images also reported high intra- and intergrader repeatability with coefficient of variation ranging from 0.33% to 0.98% and ICC ranging from 0.93 to 0.95.[11] Our high reliability indices can be explained partly by the incorporation of automated skeletonized vessel tracing in SIVA-FA, which minimizes human operator input; thus, reducing measurement variability in D_f.

Previous studies have indicated that aging and elevated blood pressure may affect the morphology of the retinal microcirculation system.[25,26] In this study, we showed that older age and hypertension were associated with lower D_{mac} and D_{disc} measurements. This was consistent with previous findings reported in white[11] and Malay[24] populations. Taken together, these findings suggested further that fractal analysis of the retinal vessels may have potential in differentiating normal and morphologically altered vascular networks. This further supports the potential application of fractal measurement in other vascular-related diseases, such as diabetes,[12,16–18,20,27] stroke,[28] congestive heart disease,[29] and chronic kidney disease.[30]

In our study we found that spherical equivalent and presence of cataract were associated with D_{mac} and D_{disc} in the univariate models, but the associations were attenuated in the multiple regression model. Li et al.[31] employed the conventional IRIS-Fractal software for D_f measurement and

Table 4. Multiple Linear Regression Analyses of Retinal Vascular Fractal Dimension With Systemic and Ocular Factors

		D_{mac}			D_{disc}		
Risk Factors	Unit Change	β (95% CI)	sβ	P	β (95% CI)	sβ	P
Age	Per 10 y	−0.03 (−0.04 to −0.02)	−0.43	<0.001	−0.03 (−0.04 to −0.02)	−0.41	<0.001
Sex	Female vs. male	−0.01 (−0.02 to 0.01)	−0.05	0.476	−0.01 (−0.03 to 0.01)	−0.09	0.232
Spherical equivalents	Per SD (1.9 D)	0.00 (−0.01 to 0.01)	0.01	0.890	0.00 (−0.01 to 0.01)	0.01	0.888
Hypertension	Presence vs. absence	−0.02 (−0.04 to −0.01)	−0.18	0.012	−0.02 (−0.04 to 0.00)	−0.18	0.021
Cataract	Presence vs. absence	−0.01 (−0.03 to 0.01)	−0.10	0.194	−0.01 (−0.03 to 0.02)	−0.04	0.671

similarly reported that spherical equivalent had insignificant influence on retinal vascular D_f. However, they reported that the presence of a cataract was significantly associated with lower retinal vascular D_f. This may indicate that retinal vascular D_f measured by SIVA-FA is less affected by media opacities, compared to the conventional IRIS-Fractal software. This could be explained potentially by the difference in the vessel detection algorithm in the two software packages: IRIS-Fractal's vessel line tracing algorithm takes into account vessel width measurement,[11] while SIVA-FA's algorithm employs a skeletonized vessel tracing method that is not affected by vessel width.

We observed that D_{mac} was higher than D_{disc} for a given participant. This is likely because the D_{mac} region includes a larger area without prominent vasculature, for example, in the foveal avascular zone where there is a paucity of retinal vessels. In addition, the retinal vessels are relatively dense around the optic disc for the smaller area defined for its measurement, leading to a larger D_{disc} compared to D_{mac}. Fractal dimension measures the ability of a fractal structure to fill space and, thus, areas such as these with lesser vessels will have a lower fractal dimension.

Compared to existing software programs used to measure D_f, such as the IRIS-Fractal[29,30,32] and the Benoit Fractal Analysis System,[33] the newly developed SIVA-FA is the first comprehensive computer-assisted program that we are aware of with a specific feature to perform semiautomated segmentation and skeletonisation of perimacular vessels, as well as analyze D_f at the perimacular region. Existing software packages that analyze macular fractal dimension require manual segmentation, which significantly increases grading time. The purpose of our software is to serve as a rapid, all-in-one tool for the calculation of macular fractal dimension without the need for manual tracing or computation. It analyses the parafoveal vascular networks in a noninvasive manner using digital fundus images, and may enhance our understanding of the relationship between macular microvascular structure and macular disease. The grading time of SIVA-FA also is reduced greatly compared to existing IRIS-Fractal program (2 vs. 5 minutes) as manual input is minimized further. Due to the increased speed and automation of this new software compared to other programs analyzing macular fractal dimension, our participant sizes are much larger compared to previous studies that involve macular fractal dimensions. Furthermore, SIVA-FA also is compatible with D_f measurements from digital fluorescein angiography images (as shown in Fig. 2), which is a feature not available in other semiautomated single software packages.

The strengths of this study include selected subjects with a wide range of systemic and ocular characteristics. In addition, standardized protocols in analyzing retinal photographs and measuring clinical parameters were used consistently in our study. However, there are a few limitations in this study. First, there are few cases of high myopia in our study. This may have affected our evaluation on the influence of spherical equivalent on D_f measurement. Further evaluation of spherical equivalent involving high myopia cases is needed. Second, there is a potential for measurement errors in images with extensive peripapillary atrophy and a tigroid fundus, where choroidal vessels may be misinterpreted by the software as retinal vessels. However, such cases were rare and were identified

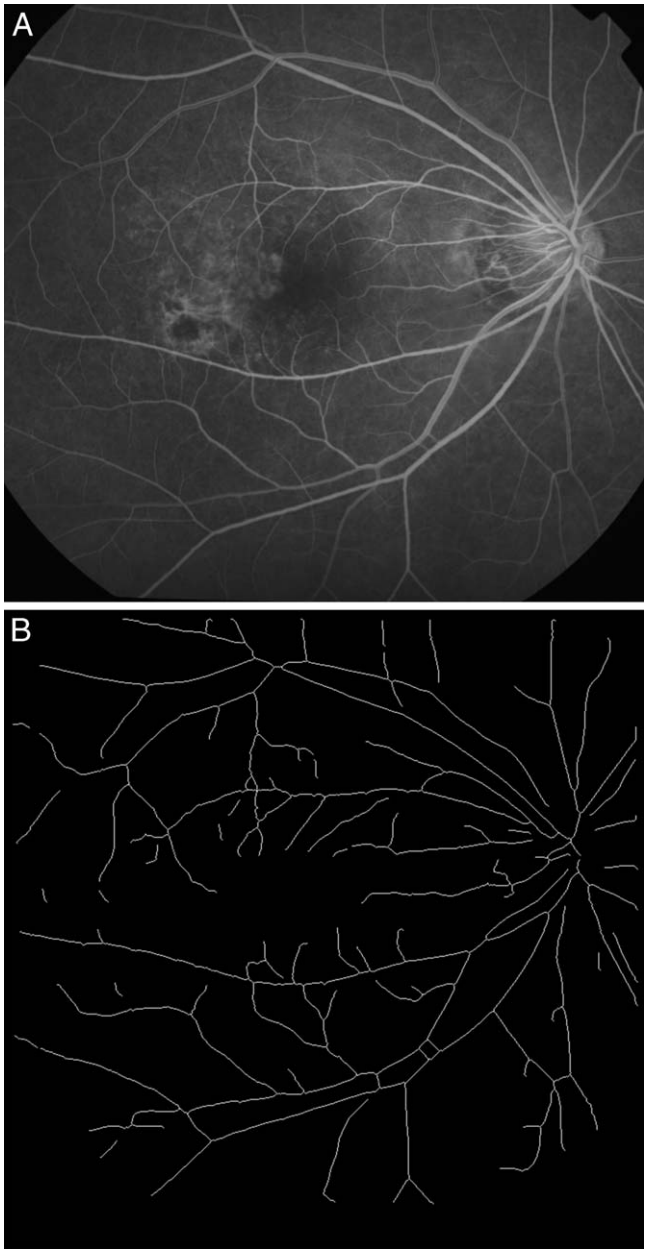

Figure 2. Fractal analysis for a fundus fluorescein angiography (FFA) image using Fractal Analyzer. SIVA-FA. (**A**) FFA Image. (**B**) Skeletonized tracing of angiogram.

easily with inspection by graders, with adjudication by a senior grader for the purposes of this study. Third, the scope of this study did not include multifractal or lacunarity analysis, which should be performed in future studies. Fourth, due to the cross-sectional nature of our study, the causal relationship between D_f and hypertension cannot be assessed definitely. Our findings may warrant further longitudinal evaluations of the causal link between D_f and hypertension. Fifth, our study was a subsample selected from a population-based study (SINDI), which may limit the generalizability of our results.

Further studies are required to validate the proposed software further. First, the robustness of the software should be tested in specific clinical cohorts, such as diabetic retinopathy and maculopathy. Second, the effect of image disturbing artefacts, such as low contrast, background noise, shadows, and lighting conditions on the software performance, also should be evaluated to validate the software. Third, previous studies[34,35] have reported high right–left eye correlation in retinal vessel caliber. However the intereye symmetry of retinal vascular fractal dimension is unknown and it should be assessed further.

In conclusion, we described a self-contained and efficient computer software package that gives excellent intragrader, intergrader, and intrasession reliability for D_f measurement in macular and optic disc-centered retinal photographs. We showed that D_{mac} is a stronger predictor of age and hypertension than D_{disc}. Fractal Analyzer may be potentially useful in the evaluation and noninvasive diagnosis of macular disease.

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