

Quantitative Assessment of Early Diabetic Retinopathy Using Fractal Analysis

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OBJECTIVE — Fractal analysis can quantify the geometric complexity of the retinal vascular branching pattern and may therefore offer a new method to quantify early diabetic microvascular damage. In this study, we examined the relationship between retinal fractal dimension and retinopathy in young individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS — We conducted a cross-sectional study of 729 patients with type 1 diabetes (aged 12–20 years) who had seven-field stereoscopic retinal photographs taken of both eyes. From these photographs, retinopathy was graded according to the modified Airlie House classification, and fractal dimension was quantified using a computer-based program following a standardized protocol.

RESULTS — In this study, 137 patients (18.8%) had diabetic retinopathy signs; of these, 105 had mild retinopathy. Median (interquartile range) retinal fractal dimension was 1.46214 (1.45023–1.47217). After adjustment for age, sex, diabetes duration, A1C, blood pressure, and total cholesterol, increasing retinal vascular fractal dimension was significantly associated with increasing odds of retinopathy (odds ratio 3.92 [95% CI 2.02–7.61] for fourth versus first quartile of fractal dimension). In multivariate analysis, each 0.01 increase in retinal vascular fractal dimension was associated with a nearly 40% increased odds of retinopathy (1.37 [1.21–1.56]). This association remained after additional adjustment for retinal vascular caliber.

CONCLUSIONS — Greater retinal fractal dimension, representing increased geometric complexity of the retinal vasculature, is independently associated with early diabetic retinopathy signs in type 1 diabetes. Fractal analysis of fundus photographs may allow quantitative measurement of early diabetic microvascular damage.

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Recent studies support the value of computer-based imaging analysis of fundus photographs to study vascular complications of diabetes (1). For example, changes in retinal vascular caliber have been associated with increased risk of diabetes and may predict the onset of diabetic microvascular and macrovascular complications (1–6).

However, previous studies have largely focused on the caliber of retinal

vessels, which represents only one of the many aspects of the retinal vascular geometry. Other structural parameters of the retinal vasculature (e.g., branching angle, vascular tortuosity) are now being explored (7,8). Although all these measures may individually convey some information regarding specific aspects of the retinal vascular network, there is a lack of a single “global” measure that can summarize the branching pattern of the

retinal vasculature as a whole. Such a measure may combine subtle vascular abnormalities and thus be a more sensitive indicator of microvascular disease.

The retinal vascular tree has a branching pattern that exhibits the property of self-similarity and is considered to be a fractal structure (9–14). Fractal analysis has been used in many branches of medicine to characterize the geometric complexity of blood vessels, including those in the eyes (9,12,14). The geometric complexity of the retinal vasculature can be quantified through calculation of fractal dimension from digital retinal images.

We have recently shown that fractal analysis of the retinal vasculature from fundus photographs can be performed reliably and efficiently using a novel computer-based program and that variations in retinal vascular fractal dimension are correlated with several biological parameters such as age and blood pressure (15). To further determine the utility of computerized retinal vascular fractal analysis in detecting microvascular complications, we examined the relationship between retinal vascular fractal dimension and diabetic retinopathy in a cohort of young individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS

This was a cross-sectional study of children and adolescents, aged 12–20 years old, with type 1 diabetes who were managed at the Children’s Hospital at Westmead, Sydney, Australia. Detailed characteristics of the study population have been reported previously (16–18). In brief, all type 1 diabetic patients, aged between 12 and 20 years, attending the Diabetes Complications Assessment clinic at the Children’s Hospital at Westmead were invited to participate in this study. The definition of type 1 diabetes was based on criteria for registration in the Australasian Pediatric Endocrine Group Diabetes Register and national guidelines. Of the 810 participants who had retinal photographs taken between 1990 and 2002, we excluded those with photographs of insufficient quality for retinopathy assessment ($n = 1$) or fractal analysis ($n = 80$) (mostly due to media opacity or image defocus), leaving 729 (90.0%) participants for this analysis.

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Table 1—Participant characteristics by retinal vascular fractal dimension quartiles

Characteristics	All	Retinal vascular fractal dimension				P value
		First quartile: <1.45023	Second quartile: 1.45023–1.46207	Third quartile: 1.46214–1.47215	Fourth quartile: ≥1.47217	
n	729	182	182	182	183	
Sex (% male)	44.2	40.7	45.6	48.9	41.5	0.722
Age (years)	13.6 (12.8–15.0)	13.6 (12.8–14.9)	13.6 (12.8–15.0)	13.7 (12.9–15.0)	13.7 (12.8–15.1)	0.724
Diabetes duration (years)	5.3 (3.4–8.1)	4.6 (3.2–7.3)	5.2 (3.3–8.0)	5.4 (3.6–8.0)	5.8 (3.5–8.6)	0.016
BMI (kg/m ²)	21.2 (19.3–23.6)	21.0 (19.3–23.8)	21.5 (19.3–23.7)	21.5 (19.4–23.7)	20.9 (19.1–23.4)	0.907
Systolic blood pressure, age- and sex-adjusted percentile	68 (46–85)	68 (52–85)	64 (50–85)	70 (42–85)	69 (43–85)	0.615
Diastolic blood pressure, age- and sex-adjusted percentile	72 (50–83)	67 (47–83)	71 (50–82)	72.5 (52–83.5)	74 (50–84)	0.241
A1C (%)	8.4 (7.7–9.3)	8.4 (7.7–9.4)	8.2 (7.7–9.2)	8.8 (7.8–9.6)	8.4 (7.7–9.1)	0.673
Total cholesterol (mmol/l)	4.3 (3.8–4.8)	4.3 (3.8–4.8)	4.3 (3.8–4.8)	4.2 (3.6–4.8)	4.3 (3.9–4.8)	0.877

Data are median (interquartile range) or proportion, as appropriate. P values relate to trend across quartiles, assessed by Kendall's rank correlation test or Cuzick nonparametric test for trend.

The characteristics of the included and excluded participants (e.g., age, diabetes duration, glycemia, and blood pressure) were similar (data not shown).

Retinal photography and retinopathy assessment

Retinal photography was performed according to a standardized protocol, as detailed elsewhere (16–18). Briefly, Early Treatment Diabetic Retinopathy Study (ETDRS) seven-standard field stereoscopic retinal photographs were taken of both eyes after pupil dilation. Diabetic retinopathy was graded from these photographs by an ophthalmologist who was masked to participants' characteristics, according to the ETDRS adaptation of the modified Airlie House classification. Retinopathy was defined as ETDRS level 21 (minimal nonproliferative diabetic retinopathy) or greater. The overall agreement, based on 30% of photographs graded independently by another ophthalmologist also masked to subject characteristics, was high (weighted $\kappa = 0.80$) (2).

Fractal analysis of retinal photographs

Fractal analysis was performed from ETDRS field 1 (centered on the optic disc) fundus photographs using a new computer-based program (International Retinal Imaging Software [IRIS-Fractal]) based on a standardized protocol (15). For each retinal photograph, a trained grader, masked to participants' characteristics, used the program to measure fractal dimension of the retinal vasculature within a predefined circular region of 3.5 optic

disc radii centered on the optic disc. After IRIS-Fractal automatically traced all of the retinal vessels within this region, the grader checked the tracing with the original photograph and removed occasional artifacts misidentified as vessels (peripapillary atrophy, choroidal vessels, pigment abnormalities, and nerve fiber reflection). The program then performed fractal analysis and calculated fractal dimension using the box-counting approach (11,12), an established method used to quantify fractal dimension for structures that are not perfectly self-similar, such as the real-life retinal vasculature (11,13). Reproducibility of measurements by IRIS-Fractal was high, with intra- and intergrader intraclass correlation coefficients ranging from 0.93 to 0.95 (15).

Risk factors and definitions

Participants underwent standardized interviews, clinical examinations, and laboratory investigations at the time of retinal photography (16–18). BMI was calculated as weight in kilograms divided by the square of height in meters. Mean arterial blood pressure (MABP) was calculated as one-third of the systolic plus two-thirds of the diastolic blood pressure. Venous blood was obtained for measurement of A1C and total cholesterol levels. Retinal vascular caliber was measured from ETDRS field 1 photographs using a computer-based program following a standardized protocol described in detail elsewhere (19–21).

Statistical analysis

We used logistic regression to determine the odds ratio (OR) for retinopathy in re-

lation to variations in fractal dimension, initially adjusting for age and sex, and additionally for diabetes duration, A1C, MABP, BMI, and total cholesterol levels. In supplementary analysis, we further adjusted for retinal arteriolar or venular calibers, which have been associated with diabetic retinopathy in previous studies (2–4), in our models. The average fractal dimension of both eyes (or the right eye if the left eye photograph was ungradable or the left eye if the right eye photograph was ungradable) was used. Retinopathy status was determined using data from both eyes when available. In models in which retinal vascular caliber measurements were included for multivariate analyses, right eye caliber measurements were used (or the left eye if the right eye calibers were ungradable). Eye-specific analyses were also performed using retinopathy and fractal dimension data from the left eyes and then repeated using data from the right eyes to assess the internal consistency of our findings. Finally, analysis using the raw (i.e., without any manual refinement) retinal fractal dimension data was done to exclude any bias from the manual part of fractal grading in our study. All analyses were performed Stata (intercooled version 9.2 for Windows; StataCorp, College Station, TX).

RESULTS— The characteristics of our study population by fractal dimension quartiles are shown in Table 1. In our population, 137 (18.8%) participants had retinopathy in at least one eye. Among these participants, 105 had mild (ETDRS level 21–30) retinopathy and 32 had moderate (ETDRS level 31–41) retinopa-

Table 2—Relationship between retinal vascular fractal dimension and diabetic retinopathy

Retinal fractal dimension	N total	Prevalence*	Age and sex adjusted†	P value	Multivariable adjusted‡	P value
Per 0.01 increase	729	—	1.33 (1.19–1.49)	<0.001	1.37 (1.21–1.56)	<0.001
Quartile 1: <1.45023	182	16 (8.8)	1.0	—	1.0	—
Quartile 2: 1.45023–1.46207	182	31 (17.0)	2.16 (1.13–4.12)	0.020	2.07 (1.03–4.18)	0.041
Quartile 3: 1.46214–1.47215	182	39 (21.4)	2.94 (1.57–5.52)	0.001	3.01 (1.53–5.94)	0.001
Quartile 4: ≥1.47217	183	51 (27.9)	4.07 (2.21–7.49)	<0.001	3.92 (2.02–7.61)	<0.001
P_{trend}			<0.001		<0.001	

Data are n (%) or OR (95% CI). *Prevalence of diabetic retinopathy in either eye. †OR (95% CI) of prevalent diabetic retinopathy (any grade compared with none in either eye) adjusted for age (continuous per 1 year increase) and sex. ‡OR (95% CI) adjusted for age (continuous per 1 year increase), sex, diabetes duration (continuous-per 1 year increase), A1C (continuous per 1 mmol/l increase), MABP (continuous per 1 mmHg), BMI (continuous per 1 kg/m² increase), and total cholesterol (continuous per 1 mmol/l increase).

thy. Fractal dimension was not normally distributed; the average fractal dimension was higher in participants with retinopathy than in those without retinopathy (median 1.46798 [interquartile range 1.45861–1.47626] compared with 1.46068 [1.44835–1.47062], respectively; $P < 0.001$).

Table 2 shows that after adjustments for age and sex, greater fractal dimension was significantly associated with increased odds of retinopathy (odds ratio [OR] 4.04 [95% CI 2.21–7.49] comparing highest to lowest quartile of fractal dimension; OR 1.33 for each 0.01 increase in fractal dimension). This association remained with additional adjustments for diabetes duration, A1C, MABP, BMI, and total cholesterol levels.

Further adjustment for retinal arteriolar or venular caliber had minimal impact on the association (OR 3.92 [95% CI 1.98–7.75] comparing highest to lowest quartile of fractal dimension with additional adjustment for arteriolar and venular caliber, 3.93 [1.99–7.76] with additional adjustment for arteriolar caliber, and 3.77 [1.94–7.34] with additional adjustment for venular caliber). In supplementary analyses, the pattern of association was similar with the use of eye-specific data (supplemental Table 1 available in an online appendix at <http://dx.doi.org/10.2337/dc08-1233>) or raw (i.e., without any manual refinement) retinal fractal dimension data (supplemental Table 2).

CONCLUSIONS— The retinal vascular tree is a complex branching structure that cannot be sufficiently described using the classical Euclidian geometric terms. Fractals, on the other hand, represent a type of non-Euclidian geometric shape that has been applied in many aspects of medicine to describe similarly

complex branching biological structures (e.g., coronary arterioles or pulmonary bronchi). Fractals can be defined as geometric patterns whose parts resemble the whole (i.e., the property of self-similarity), and fractal dimension is a measure of the geometric complexity of a spatial object. The retinal vascular tree is a fractal object (9–14). Fractal analysis of the retinal vasculature has been performed in previous studies that provided insights into the embryology and development of retinal vessels (13).

In this study, we found that increased fractal dimension of the retinal vasculature, reflecting increased geometric complexity of the retinal vascular branching pattern, is associated with early retinopathy signs in young individuals with type 1 diabetes. This association was strong, graded, and independent of conventional retinopathy risk factors (e.g., diabetes duration or A1C).

Most of the previous studies on the relationship of structural retinal vascular changes and diabetic retinopathy focused on the assessment of retinal vascular caliber (2–4), which represents only one of many parameters of the retinal vascular geometry. Retinal vascular caliber does not convey information regarding the complexity of the retinal vascular branching pattern. Fractal dimension, on the other hand, is a “global” measure of the overall geometric complexity of the retinal vascular network (7,10). Our study suggests that fractal analysis of fundus photographs may allow quantitative measurement of early diabetic microvascular damage.

Although there have been previous attempts to study fractal dimension of the retinal vasculature (7,9,10,13), only a few small studies examined the relationship of fractal dimension and retinopathy, and most of these studies used imprecise

methods to quantify fractals. For example, in a study of 50 diabetic patients, fractal dimension was reported to be higher in those with than without proliferative retinopathy (22,23). These earlier studies were based on manual tracing of the retinal vessels, which could take up to 20 h to process a single retinal image (13). This time contrasts with our computer program, which requires only 5–10 min per image with minimal operator input, allowing application in large-scale epidemiological studies and potentially in clinical settings. The pathophysiological mechanisms underlying our findings remain unclear. In the context of vascular branching patterns, fractal dimension describes how thoroughly the pattern fills two-dimensional spaces. Therefore, it makes intuitive sense that fractal dimension should increase as new vessels form in the process of neovascularization in proliferative diabetic retinopathy, as shown previously (22,23). Nevertheless, there were no cases of proliferative retinopathy in our study of young patients with type 1 diabetes. This result suggests that the association of increased fractal dimension and retinopathy is probably not a result of proliferative changes. Importantly, it also supports our hypothesis that retinal fractal dimension may be a sensitive indicator of early retinal microvascular damage in diabetes, even before the onset of proliferative new vessels. It has been suggested that variations in fractal dimension may reflect arteriovenous differentiation after hypoxic cues during embryonic development of the retinal vasculature (13,24). Thus, fractal dimension in childhood and early adulthood may similarly reflect changes in arteriolar and venular network geometry in response to hypoxic stimuli to increase blood flow in diabetes. Clearly, additional studies are needed to verify our hypotheses.

Our study has potential clinical implications. Young patients with type 1 diabetes all have a significant lifetime risk of blindness from diabetic retinopathy, and there is a need to improve the identification and monitoring of this sight-threatening eye disease throughout the lives of these patients. This remains clinically challenging because of, at least in part, the limited number of specific risk factors for retinopathy in young diabetic patients. As shown in our study, increased retinal vascular fractal dimension may represent a novel and specific biomarker for the presence of early diabetic retinopathy, even after factoring in the effects of traditional retinopathy risk factors (e.g., diabetes duration or A1C). Thus, computer-based fractal analysis of retinal images may offer a new means to quantitatively assess the risk of diabetic retinopathy and may provide additional information regarding the risk of other diabetes microvascular complications. Additional studies, including prospective studies, are needed to elucidate the role of fractal analysis in the clinical assessment and prediction of major outcomes of diabetic patients.

Strengths of our study include a large sample of young patients with type 1 diabetes, quantitative evaluation of fractal dimension, standardized assessment of diabetic retinopathy using stereoscopic seven-field retinal photographs, and detailed assessment of potential confounding variables (e.g., diabetes duration, A1C, and retinal vascular caliber). Limitations should also be noted. First, the cross-sectional design of the study did not allow us to ascertain whether the greater fractal dimension is antecedent or consequential to diabetic retinopathy. Additional studies, which are currently underway, are needed to determine the temporal sequence of the observed association. Second, there are several potential sources of error in fractal dimension measurement, as described previously (15). In particular, falsely high fractal dimension might result from the inclusion of nonvascular artifacts or large retinopathy lesions. However, most of these artifacts would have been eliminated through manual inspection by our grader. Only 4 of the 137 patients with retinopathy in our study had retinopathy signs in ETDRS field 1 photographs in which fractal dimension was measured. All of these lesions were of small size (dot hemorrhage or microaneurysms) and were not traced by the program. Exclusion of these four participants from our analysis did not al-

ter our results (data not shown). Moreover, random measurement errors are likely to create noise and thus bias the results toward the null. The positive and strong associations and the substantially high reproducibility of measurements provide reassurance that artifacts are probably not a major confounding issue in our study. Finally, the relationship of fractal dimension and proliferative retinopathy remains to be determined, as we had no cases of such severe retinopathy in our patient cohort.

In summary, our study shows that greater retinal vascular fractal dimension, reflecting increased geometric complexity of the retinal vasculature, is strongly associated with diabetic retinopathy signs in type 1 diabetes, independent of standard retinopathy risk factors. If our findings are confirmed in other samples and in prospective studies, fractal analysis of fundus photographs using a semiautomated computer-based program may be applied to clinical studies and even clinical trials evaluating risk factors and novel treatments for early microvascular disease. Further research is needed to investigate the clinical utility of retinal fractal analysis as a potential tool to quantitatively assess early diabetic microangiopathy.

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