Fractal analysis of region-based vascular change in the normal and non-proliferative diabetic retina

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Abstract


Methods. Binary (black/white) vascular patterns of the human retina originating at the optic disc were obtained by semi-automatic computer processing of digital images from 60-degree fundus fluorescein angiography of 5 normal eyes and 5 eyes with non-proliferative diabetic retinopathy (NPDR). As determined by image resolution, vascular patterns included vessels with diameters ≥ 50 μm and excluded small vessels and capillaries. The density of linearized (i.e., skeletonized) vascular patterns in the macular region versus paramacular region (termed “region-based” linearized vascular pattern) was quantified with the fractal dimension (Df) and confirmed by grid intersection (ρv).

Results. By region-based quantification, Df and ρv were significantly higher in the normal macular region than in the NPDR macular region (p = 0.008 and p = 0.019, respectively). However, differences in Df and ρv between the normal and NPDR paramacular regions were not strongly significant (p = 0.168 and p = 0.337, respectively).

Conclusions. Results from the retrospective analytical study demonstrate the feasibility of using quantitative region-based fractal analysis of early-stage vascular disease in the human retina. The results are encouraging for a broader study of diverse patient populations.

Keywords: fractal; region-based; vascular; retina; image analysis; non-proliferative diabetic retinopathy

Introduction

The goal of this study was to investigate the effectiveness of fractal analysis for quantitative characterization of the vascular patterns that are altered during the early stages of human retinal disease. We therefore obtained binary (black/white) vascular patterns originating at the retinal optic disc (Fig. 1) by computer processing of images from 60° clinical fluorescein angiography of normal patients or patients diagnosed with non-proliferative diabetic retinopathy (NPDR). As determined by spatial resolution of the angiography, capillaries and smaller vessels were excluded from this retrospective fractal analysis. The image-extracted vascular patterns used for the novel region–based analysis contained only arteries and veins of diameter ≥ approximately 50 μm.

Preliminary qualitative observations of the linearized (skeletonized) vascular patterns in the normal and NPDR macula suggested that vascular morphology had already changed by this relatively early stage of retinal disease (Fig. 2). For the region-based quantification of linearized vascular pattern, macular and paramacular regions were defined geometrically. The linearized vascular pattern in each region was quantified by two different statistical measures of complex
Figure 1. Image processing of fluorescein angiograms. Film negatives from 60° fluorescein angiograms photographed at 1.7× magnification at the arterio-venous stage were (A) scanned as gray scale, digitized images of 1024 pixel × 1024 pixel. The gray scale image, such as the NPDR image shown here, was converted by semi-automatic computer processing into (B) a binary image and (C) a linearized (skeletonized) image. Retinal zones defined for region-based morphometry include superior (1), superotemporal (2), macular (3), temporal (4), inferior (5) and inferotemporal (6). The paramacular region includes sub-regions or zones (1), (2), (4), (5) and (6).

Figure 2. Region-based fractal analysis of linearized vascular pattern in the normal and NPDR retina. Inspection of binary and linearized images of vascular pattern extracted from representative fluorescein angiograms of the normal retina (A, B) or the NPDR retina (C, D) suggests that vessel density was greater in the normal macular region than in the NPDR macular region. In the normal retina (C), the fractal dimension \( D_f \) and grid intersection \( r_v \) were greater in the macular region (1.49 and 177, respectively) than in the paramacular region (1.38 ± 0.05 and 110 ± 23). In the NPDR retina (D), \( D_f \) and \( r_v \) were essentially equivalent in the macular and paramacular regions (1.39 and 112, respectively, compared to 1.40 ± 0.01 and 118 ± 11).
spatial pattern and density, the fractal dimension \(D_f\) and grid intersection \((\rho_g)\). The subject of the current study, the branching vascular tree in the superficial posterior retina, is complementary to, but distinct from, a previous study of perifoveal capillaries quantified by other morphological parameters in human fluorescein angiograms.2 Previous reports of \(D_f\) in the diabetic versus normal human retina are reviewed in Discussion.3–12 Results reported here suggest that region-based fractal analysis is a new, effective method for evaluating the early vascular progression of diabetic retinopathy.

Fractal geometry, which is common in nature, is manifested in the complex spatial patterns of objects such as trees, coastlines and snowflakes, and in complex spatiotemporal phenomena such as vascular-based physiological scaling.13–17 Self-similarity, the key concept underlying the non-Euclidean (i.e., nonintegral) dimensionality of fractals, is the geometric property whereby the spatial pattern of an object remains constant despite change of scale or magnification. The fractal dimension, \(D_f\), of a self-similar object such as a branching vascular tree is a physically meaningful measure of altered pattern or branching density. We previously used fractal analysis as a sensitive measure of small changes in boundaries of the retinal vessels filled with fluorescein dye are somewhat obscured by choroidal fluorescence (both diffuse and linear), retinal light reflexes and in the case of NPDR, by intraretinal microaneurisms, exudates and hemorrhagic leakage of the dye. However, several characteristics of retinal blood vessels in the fluorescein angiogram served as the basis for a vessel extraction algorithm which is reported elsewhere.22 Vessel characteristics include: a) relatively dark intensity (on the photographic negative), b) uniform intensity (i.e., low intensity variance), c) considerable contrast with surrounding background structures, d) a branching pattern composed of linear shape elements, and e) a characteristic intensity profile in the cross section. The computer algorithm was applied to a subset of gray scale images for the accurate extraction of several generations of retinal vessels. Vessels which were not clearly outlined in the computer-processed image were traced manually from the original gray scale image using the layering capabilities of Adobe Photoshop, and were evaluated by two independent observers as described above.

Following final selection of vasculature, the image was binarized by visual inspection, rescaled to support fractal analysis (described below) and linearized (i.e., skeletonized) by the automatic skeletonizing tool of NIH Image (Fig. 1B). Retinal vessels obtained from a fluorescein angiogram were therefore converted by image processing into a linear vascular pattern originating from the optic disc. Optical distortion resulting from fundus photography was not corrected, but errors in fractal measurements due to retinal curvature are small (approximately 3%).4

Methods

Fluorescein angiography

For this retrospective analytical study, we randomly selected fundus fluorescein angiograms of acceptable image quality from 5 normal eyes (4 patients) and 5 eyes (5 patients) diagnosed with mild to moderate NPDR previously photographed at the Ophthalmology Clinic, University of Washington at 1.7× magnification with a 60 degree fundus camera (60-UV, Canon Optical, Japan). All angiograms were compatible with fundus appearance. For each eye, one frame (negative) was chosen from the arterio-venous stage of the angiogram when the entire retinal vascular tree was maximally filled with fluorescein, before any evidence of fluorescein leakage occurred (i.e., approximately 21–25 seconds after injection). Frames with similar orientation of the major vascular arcades in the optic disc, macula, and inferior, superior and temporal zones were selected. Informed consent for fluorescein angiography had been obtained previously from each patient, and study of the angiograms was performed with the approval of the University of Washington Human Subjects Research Committee.

Imaging

The original negative (3.6 cm × 2.4 cm) was scanned with a film scanner (LS – 4500AF, Nikon, Japan) as a digital gray scale image at 2× magnification (7.1 cm × 4.8 cm) and a resolution of 236 pixel/cm (image resolution was therefore approximately 50 μm). The central region of 1024 pixel × 1024 pixel was extracted as a gray scale image (Fig. 1A). The ten gray scale images were binarized to black/white by a combination of (1) manual processing in NIH Image 1.56–1.57 and Adobe Photoshop 4.0–5.0 and (2) computerized processing (described below). With a methodology similar to those described in earlier reports,9,10,21 the final processed selection of all retinal vasculature was performed by two independent observers experienced in the assessment of vascular morphology.

Automatic computerized extraction of retinal vasculature from a gray scale fluorescein angiogram is difficult, because boundaries of the retinal vessels filled with fluorescein dye are somewhat obscured by choroidal fluorescence (both diffuse and linear), retinal light reflexes and in the case of NPDR, by intraretinal microaneurisms, exudates and hemorrhagic leakage of the dye. However, several characteristics of retinal blood vessels in the fluorescein angiogram served as the basis for a vessel extraction algorithm which is reported elsewhere.22 Vessel characteristics include: a) relatively dark intensity (on the photographic negative), b) uniform intensity (i.e., low intensity variance), c) considerable contrast with surrounding background structures, d) a branching pattern composed of linear shape elements, and e) a characteristic intensity profile in the cross section. The computer algorithm was applied to a subset of gray scale images for the accurate extraction of several generations of retinal vessels. Vessels which were not clearly outlined in the computer-processed image were traced manually from the original gray scale image using the layering capabilities of Adobe Photoshop, and were evaluated by two independent observers as described above.

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Region-based vascular morphometry

The fractal dimension \(D_f\) and grid intersection \((\rho_g)\) were measured in linearized vascular images in order to measure only
the space-filling linear extension of the vessels, and not the thickness of the vessels. Df was estimated with a computer program implementing the method of box-counting. By this method, the image is overlaid with series of square boxes of decreasing size (s = 512, 256, 128, . . . 1, where s denotes a single pixel), and the number of boxes [N(s)] containing at least one black pixel is counted. The negative value of the least squares regression slope of the plot of log N(s) versus log s yields Df. For all images, the regression slope of box-counting plots was highly linear (r² ≥ 0.97). A computer program implementing grid intersection counted the number of intersections (ρv) between vessels in a linearized image and a superimposed rectangular grid.

For region-based vascular morphometry, vessel density was measured by Df and ρv, separately in the central macular region and adjoining paramacular region. The binary image of the entire posterior retina (1024 pixel × 1024 pixel) was subdivided into nine equal zones of 341 pixel × 341 pixel, with the central zone positioned on the macula (Fig. 1C). The macular region and 5 paramacular sub-regions or zones located superior, superotemporal, temporal, inferotemporal and inferior to the macula (Fig. 1C) were rescaled from 341 pixel × 341 pixel to 256 pixel × 256 pixel (to accommodate the binary fractal algorithm) and linearized. A grid size of 32 pixels was used for ρv. Vessel density in the overall paramacular region was calculated as the mean value of Df or ρv, measured for the 5 sub-regions (Fig. 1C). We also analyzed vessel density by Df and ρv in the entire retinal image. For this analysis, the binary retinal image was rescaled by 50% to 512 pixel × 512 pixel (to accommodate computer requirements) and linearized. Each data point for Df or ρv (with a grid size of 64 pixels) represents the mean and standard deviation (SD) of measurements of 5 retinas (normal or NPDR), and p-values were calculated by a two-tailed, heteroscedastic Student’s t-test.

Results

Results of the region-based fractal analysis of linearized vascular patterns extracted from angiograms of the normal and NPDR human retina are summarized in Figs. 2–3. Two major differences between the normal and NPDR retina were qualitatively apparent (Fig. 2): (1) vessel density in the normal macular region (Fig. 2A, B) appeared greater than vessel density in the NPDR macular region (Fig. 2C, D) and (2) vessel density in the normal macular region (Fig. 2A, B) appeared greater than in the normal paramacular region. These observations (Fig. 2) were confirmed by subsequent quantitative measurements (Fig. 3).

In the normal macular region, mean ± SD of Df and ρv was 1.46 ± 0.02 and 152 ± 23, respectively, compared to 1.41 ± 0.02 and 120 ± 10 in the NPDR macular region (for Df and ρv, p = 0.008 and p = 0.019). Vessel density in the normal macular region was significantly higher than in the normal paramacular region, in which Df and ρv were 1.37 ± 0.02 and 105 ± 6 (p = 0.001 and p = 0.006, respectively). In contrast, differences between vessel density in the normal paramacular region and NPDR paramacular region, for which Df and ρv were 1.40 ± 0.02 and 113 ± 12, were not strongly significant (p = 0.168 and p = 0.337, respectively). Vessel density in the NPDR retina as measured by Df and ρv did not differ significantly between the macular and paramacular regions (p = 0.284 and p = 0.594, respectively).

As shown in Figure 4, vessel density in linearized vascular patterns extracted from the entire (undivided) binary images of normal and NPDR retinas also did not differ significantly. In the normal retina, Df and ρv were 1.55 ± 0.01 and 308 ± 16 and in the NPDR retina, 1.57 ± 0.03 and 319 ± 19 (p = 0.474 and p = 0.590, respectively).

Figure 3. Region-based fractal analysis of the normal and NPDR retina. With region-based morphometry, vessel density in linearized vascular patterns extracted from the normal retina as measured by (A) the fractal dimension (Df) or (B) grid intersection (ρv) was greater in the macular region than in the paramacular region (mean and SD for n = 5; p = 0.001 and p = 0.006 for Df and ρv, respectively). This region-based difference in vessel density was not preserved in NPDR retinas, where vessel density did not differ significantly in the macular and paramacular regions (p = 0.284 and p = 0.594, respectively). Vessel density in the normal macula as measured by Df and ρv was therefore greater than in the NPDR macula (p = 0.008 and p = 0.019, respectively).
Discussion

By quantitative region-based fractal analysis of linearized vascular pattern, greater vessel density was measured in the normal macula than in the NPDR macula. The results demonstrated surprisingly strong statistical confidence and reproducibility, given the limited sampling number of this retrospective analytical study (for n = 5, p = 0.008 and 0.019 by Df and rv). Furthermore, vessel density in the normal macular region was greater than in the normal and NPDR paramacular regions (by Df, p = 0.001 and 0.002 and by rv, 0.006 and 0.012, respectively). In an experimental model of vascular change in vivo, region-based fractal analysis was used previously to measure vessels of the quail chorioallantoic membrane (CAM).18–20 As in the human retina, fractal measurements of change in the complex linearized vascular pattern of the CAM was highly sensitive and reproducible. Region-based fractal analysis of linearized vascular patterns in the human retina may be useful for the sensitive, reproducible detection of early vascular change in retinal disease, as well as for providing insight into the progression of retinal disease processes. For clinical applications, however, confidence in the quantitative fractal results for the NPDR retina would require confirmation by a more comprehensive clinical study.

At the level of image resolution described here (approximately 50μm), the space-filling density of the entire superficial vascular tree throughout the posterior pole of the human retina was equivalent in the NPDR and normal retina (Fig. 4), as measured in linearized vascular patterns by the fractal dimension (Df) and confirmed by grid intersection (rv). This result is in agreement with established criteria for NPDR.23 However, region-based fractal analysis suggests that vessel density decreased in the NPDR macula relative to the normal macula and became relatively equivalent to vessel density in the normal and NPDR paramacular regions. Decreased vessel density in the NPDR macula measured in this study correlates positively with results reported previously for decreased perifoveal capillary density in NPDR.1,2

Alterations in retinal vasculature during early-stage diabetic retinopathy (i.e., by NPDR) measured by Df and rv may have resulted from several disease mechanisms and/or imaging artifacts. For example, decreased vessel density in the NPDR macula may have resulted from non-perfusion, drop-out and/or narrowing of vessels. There is no indication that vessels were obscured by fluorescein leakage, because the selection of angiograms at the maximum arteriovenous stage of fluorescein filling excluded images of fluorescein leakage acquired at later stages of angiography. Measurements of decreased vessel density in the NPDR macula could have resulted from opacity of the cornea or lens, which might be expected to obscure vessels in NPDR. Arguing against the interpretation of measurements in the NPDR macula as artifact due to anterior opacification is the fact that equivalent vessel density was measured in the normal and NPDR paramacular regions. However, more complete studies would be required to confirm the measurements of macular change in NPDR.

Fractal analysis suggests that the space-filling properties of individual region-based vascular patterns are highly similar within each clinical population (i.e., normal or NPDR retina), because variation among the samples of each population was low (Figs. 3–4). Even at the relatively low imaging resolution of fluorescein angiography, the fractal dimension (Df) may elucidate the significance of biological change during early-stage disease in which space-filling dimensionality is of fundamental importance. A linearized (skeletonized) vascular image is a direct representation of total vessel length and in general, altered vessel density is measured more sensitively by Df in linearized images than in binary images.18,19 The sensitivity of Df to vascular change in a linearized image may result from a greater dependence of vascular remodeling on increases in total vessel length than on changes in other morphological parameters such as vessel diameter.19 An advantage of images acquired at low magnification, such as the
images in this study, is that in general the fractal analysis of branching structure can be applied to larger branching areas of the arterial and venous trees.

Interestingly, $D_f$ increased in linearized vascular images in the rapidly developing CAM of the quail embryo as a highly first-order function of time.\textsuperscript{18} Inhibition of angiogenesis by angiostatin\textsuperscript{18} and transforming growth factor-$\beta_1$ (TGF-$\beta_1$)\textsuperscript{19} has been measured in linearized images by $D_f$ and confirmed by $\rho_v$, as was angiogenic stimulation by fibroblast growth factor-2 (FGF-2 or bFGF), which correlated positively with change in FGF tyrosine kinase receptor density.\textsuperscript{20} Change of vessel density in linearized images has also been measured by the box-counting method for $D_f$ in the developing chicken CAM\textsuperscript{24} and in tumor vasculature in the mouse, for which vascular heterogeneity in tumor tissue was further characterized by percolation analysis.\textsuperscript{17,26,27} Fractal algorithms are used frequently in computer graphics to generate images that strongly resemble their natural counterparts. Estimators or measures of $D_f$ for fractal objects observed in nature, including the box-counting estimator of $D_f$ employed by us, are generally based on homogeneous power-law analyses that preserve exponents throughout rescaling (i.e., exponents remain constant).

Fractal analysis has been used previously to measure vessel density in retinal fundus photographs and fluorescein angiograms of human clinical subjects, especially for the quantification of progression in diabetic retinopathy.\textsuperscript{3–12} However, these reports are few in number and results are rather contradictory, apparently due to considerable variation in both imaging technique and the mathematical method of fractal analysis. Most analyses relied on the manual tracing of projected angiograms.\textsuperscript{3,7,9–11,21} By the two-point correlation method for estimation of $D_f$, the vessel density of normal and PDR retinas differed significantly,\textsuperscript{8} but was indistinguishable by the box-counting method.\textsuperscript{7} Using a local tangential slope analysis, others have questioned whether the vascular and neural trees of the retina are fractal.\textsuperscript{12}

Results in the present study are consistent with the argument that retinal vasculature is indeed fractal because the vascular patterns are statistically self-similar over a range of length scales, the most common test for fractal structure.\textsuperscript{3,4,8–10} We attribute the successful quantification by $D_f$ and $\rho_v$ of vascular change in early diabetic retinopathy (NPDR) to: (1) recent progress in image-scanning technology and (2) the region-based analysis of linearized vascular pattern. While the results are encouraging, the fractal method needs to be confirmed using an expanded population of individuals. Improvements in imaging resolution can be expected to improve the sensitivity of region-based fractal analysis for clinical research.

In conclusion, quantification of linearized vascular patterns in the normal and NPDR retina indicates that region-based fractal analysis has the potential to become a valuable tool for research in the human retina and perhaps a reproducible method for quantification of vascular change in the clinical monitoring of retinal disease.

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