Retinal Vascular Fractal Dimensions and Their Association with Macrovascular Cardiac Disease

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Keywords

Coronary artery bypass grafting · Retinal vascular geometry · Fractals · Imaging

Abstract

Introduction: As the only part of the human vasculature, the retina is available for direct, noninvasive inspection. Retinal vascular fractal dimension (D_F) is a method to measure the structure of the retinal vascular tree, with higher noninteger values between 1 and 2 representing a more complex and dense retinal vasculature. Retinal vascular structure has been associated with a variety of systemic diseases, and this study examined the association of D_F and macrovascular cardiac disease in a case-control design. **Methods:** Retinal fundus photos were captured with Topcon TRC-50X in 38 persons that had coronary artery bypass grafting (CABG, cases) and 37 cardiovascular healthy controls. The semiautomatic software VAMPIRE was used to measure retinal D_F . **Results:** Patients with CABG had lower D_F of the retinal main venular

vessels compared to the control group (1.15 vs. 1.18, $p = 0.01$). In a multivariable regression model adjusted for gender and age, eyes in the fourth quartile with higher D_F were less likely to have CABG compared to patients in the first (OR, 7.20; 95% confidence interval: 1.63–31.86; $p = 0.009$) and second (OR, 8.25; 95% confidence interval: 1.70–40.01; $p = 0.009$) quartiles. **Conclusions:** This study demonstrates that lower complexity of main venular vessels associates with higher risk of having CABG. The research supports the hypothesis that the retinal vascular structure can be used to assess nonocular macrovascular disease.

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Introduction

The vessels of the retinal circulation are uniquely available for direct in vivo inspection. Growing evidence in various features from retinal vessel examinations, such as vessel caliber [1–6], retinal vascular fractal dimension

(D_F) [7, 8], and microvascular abnormalities [9, 10], suggests that retinal structural measurements can be used to detect, or even predict, cardiovascular disease.

The concept of statistical self-similarity introduced by Mandelbrot [11] in 1967 stated that a fraction of a pattern statistically can be considered the whole. In nature, this can be applied to coastal lines, snowflakes, and lightning, as well as the retinal vascular tree [12]. D_F can be measured as a noninteger between 1 and 2. The higher the number, the more the complexity and density are represented in the retinal vascularity [13].

Macrovascular cardiac disease is traditionally described as dysfunction of the larger peripheral coronary arteries, but increasing evidence points to microvascular dysfunction as a pathogenic to macrovascular coronary artery disease [14, 15]. D_F differs from other more simplified retinal vessel assessments as, for example, vessel caliber that measures only a small part of bigger retinal vessels. D_F summarizes the full pattern of the retinal circulation into a single measurable component including the smaller and bigger retinal vessels. Methods to directly measure the global coronary circulation are lacking [15], but D_F has been investigated as a surrogate measure [7, 16, 17].

Cardiovascular disease still remains the top cause of mortality worldwide, and early risk stratification to identify and manage the diseased is essential [18]. This study aimed to investigate D_F in a population with established macrovascular cardiac disease (coronary artery bypass grafting [CABG] patients) compared to cardiovascular healthy controls.

Materials and Methods

Study examinations were performed from March 2018 until ultimo December 2018 at Odense University Hospital (OUH), Odense, Denmark. The study population consisted of a group suffering from macrovascular cardiac disease (43 cases) and a cardiovascular healthy group (42 controls).

Cases were recruited at the Department of Cardiac, Thoracic and Vascular Surgery, OUH, where they underwent CABG. Recruitment took place 4 days after surgery and the clinical examinations when they had pace wires and heart monitoring scope discontinued 4 to 6 days after surgery. Controls were recruited at the Department of Ophthalmology, OUH, on the day after they underwent cataract surgery.

Exclusion criteria included mental inability, age under 18, retinal disease (i.e., diabetic retinopathy or hypertensive retinopathy), and the need for resurgery applicable for both groups. Cases had to be CABG patients and were excluded if they had CABG combined with cardiac valve surgery. Controls could not have any history or presence of cardiac disease. Controls were not excluded if they had presence of cardiac risk factors such as hypertension, hy-

Fig. 1. A disc-centered fundus photography processed for analysis of fractal dimension by using the VAMPIRE software (Vessel Assessment and Measurement Platform for Images of the REtina; The Vampire Group, Edinburgh, United Kingdom). The blue and red vessels represent venules and arterioles, respectively. The coordinate system surrounding the optic disc is marked with zones A, B, and C located 0.0–0.5, 0.5–1.0, and 0.5–2.0 disc diameters from the optic disc center.

percholesterolemia, diabetes, and history of smoking, or if they were treated with anticoagulants from noncardiac causes.

Clinical characteristics were gathered from a full medical interview in addition to information from the patient records. After 10 minutes of rest, blood pressure was measured and standardized as a mean of 3 measurements on the right upper arm (Omron M6 AC, Kyoto, Japan). Blood samples were collected for analysis including cholesterol profile, triglycerides, and hemoglobin A1c. The Topcon TRC-50X (Topcon, Tokyo, Japan) was used to capture a 45° optic disc-centered fundus photography from the right eye (or left eye, if the right was not eligible for inclusion) after mydriasis was achieved with tropicamide 1% and phenylephrine 10%.

The VAMPIRE (Vessel Assessment and Measurement Platform for Images of the REtina; The Vampire Group, Edinburgh, United Kingdom) software was used for D_F analysis based on a prespecified protocol. The grader was not blinded to the group allocation of the individual participant. The method for the semiautomatic software is outlined in detail elsewhere [19, 20] and will only be briefly explained here.

Retinal landmarks were automatically detected, including the center and outer diameter of the optic disc and the macula center. This enabled the establishment of a coordinate system with circular zones A (0–0.5 disc diameters from the optic disc center), B (between 0.5 and 1.0), and C (between 0.5 and 2.0) around the optic disc (Fig. 1). Vessels were automatically traced as arterioles or venules,

Fig. 2. A retinal image (a) and its corresponding skeletonized image (b) illustrating the refined line tracing of the retinal vasculature.

ranging from zone A and peripheral to zone C. This creates a skeletonized pattern (Fig. 2b) of the entire retinal circulation (Fig. 2a). The automatic localization of the optic disc, macula, and vessels was not always on point, and manual corrections were made when needed. In some cases, traced vessels were erased when it was unclear which vessel type was represented and when VAMPIRE misinterpreted an artefact or the underlying choroid layer as a retinal vessel.

The software ran the final analysis by the box-counting method [19, 21, 22] developed for self-similar patterns that are not completely self-similar. Those sequences of the arterioles and venules coursing through zone C are included to calculate D_F . Outcomes were arteriolar, venular, and total D_F from all vessels traced in zone C as well as D_F from the main vessels alone. The method is validated with a high intergrader reproducibility [19].

Continuous data are presented as medians and categorical data as percentages. The 0.05 level was used to test for all statistical differences using Stata Intercooled 16 (StataCorp, College Station, TX, USA).

Differences between the 2 groups were tested by the Mann-Whitney U test used for continuous data and the Pearson χ^2 used for categorical data. A multivariable logistic regression model with adjustment for gender and age was used to display the odds ratio of macrovascular cardiovascular disease in relation to main venular fractal dimension divided into 4 quartiles.

Results

The exclusion process led to 10 exclusions leaving 75 participants for further analysis (37 controls and 38 cases). Eight were excluded due to poor image quality, one due to former central retinal vein occlusion, and one because of former cardiac disease (control group).

Differences between the 2 groups are shown in Table 1. The case group consisted of more men (76.3 vs. 51.4%, $p = 0.02$), had a higher BMI (28.0 vs. 25.5, $p = 0.02$), and were more likely to have diabetes mellitus (34.2 vs.

8.1%, $p = 0.01$), but had a lower systolic (138 vs. 143 mm Hg, $p = 0.01$) and diastolic (70 vs. 90 mm Hg, $p < 0.001$) blood pressure. Cases were more commonly medicated with antihypertensives (89.5 vs. 51.4%, $p < 0.001$), statins (94.7 vs. 29.7%, $p < 0.001$), and anticoagulants (100 vs. 21.6% $p < 0.001$).

We found no differences between the 2 groups in age, smoking history, HDL-cholesterol, triglycerides, and HbA1c. As compared to eyes of control patients, eyes of cases had lower retinal fractal dimension regarding the main venules (1.15 vs. 1.18, $p = 0.01$), but did not differ according to other retinal fractal measurements (Table 1).

Table 2 displays the odds ratio of having CABG in relation to the main venular fractals. The VAMPIRE software was unable to calculate the main venular D_F in one of our cases. In the multivariable analysis adjusted for gender and age, eyes of patients with the highest values in D_F (fourth quartile) were less likely to have been operated for CABG as compared to the eye of patients in the first (OR, 7.20; 95% confidence interval: 1.63–31.86; $p = 0.009$) and second (OR, 8.25; 95% confidence interval: 1.70–40.01; $p = 0.009$) quartiles. In fact, the risk of CABG increased with 83% for each standard deviation decrease in main venular fractal dimension (OR, 1.83; 95% confidence interval: 1.07–3.13; $p = 0.03$).

Discussion

The present study demonstrated an association between CABG and retinal main venular D_F when compared to cardiovascular healthy controls. In particular, eyes of patients from the 2 lowest quartiles compared to

Table 1. Clinical and demographic characteristics of eyes of patients operated with CABG (cases) as compared to eyes from cardiovascular healthy controls

	Cases (<i>n</i> = 38)	Controls (<i>n</i> = 37)	<i>p</i> value
Gender, men, %	76.3	51.4	0.02
Age, years	70.5	72.0	0.28
Body mass index, kg/m ²	28.0	25.5	0.02
Systolic blood pressure, mm Hg	138	143	0.01
Diastolic blood pressure, mm Hg	70	90	<0.001
History of smoking, %	71.4	78.3	0.48
Diabetes mellitus, %	34.2	8.1	0.01
Medication, %			
Antihypertensive	89.5	51.4	<0.001
Cholesterol-lowering	94.7	29.7	<0.001
Anti-coagulation	100	21.6	<0.001
Plasma levels			
Total-cholesterol, mmol/L	3.4	5.6	0.01
HDL-cholesterol, mmol/L	1.3	1.5	0.18
LDL-cholesterol, mmol/L	1.7	3.4	0.02
Triglycerides, mmol/L	1.4	1.4	0.37
HbA1c, mmol/mol	37.0	37.5	0.85
Vessel geometry			
Arteriolar fractal dimension	1.24±0.05	1.23±0.05	0.45
Venular fractal dimension	1.21±0.05	1.22±0.04	0.44
Total fractal dimension	1.39±0.04	1.38±0.03	0.47
Arteriolar fractal dimension main vessels	1.15±0.06	1.15±0.08	0.90
Venular fractal dimension main vessels	1.15±0.06	1.18±0.05	0.01*
Total fractal dimension main vessels	1.32±0.06	1.33±0.07	0.35

Data are presented as medians. Tested for differences between cases and controls. *p* values <0.05 are regarded statistically significant. History of smoking = former or current; antihypertensive medication = all kinds of antihypertensive drugs; CABG, coronary artery bypass grafting; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; HbA1c, glycosylated hemoglobin. * Statistically significant.

Table 2. Association of retinal main venular fractal dimensions and coronary artery bypass surgery in a model adjusted for age and gender

Retinal main venular fractal dimension	<i>N</i>	Cardiovascular disease
Per SD decrease, 0.0555		1.83 (1.07–3.13)*
First quartile, 1.017–1.123	20	7.20 (1.63–31.86)*
Second quartile, 1.124–1.162	18	8.25 (1.70–40.01)*
Third quartile, 1.164–1.200	18	1.79 (0.39–8.15)
Fourth quartile, 1.201–1.324	18	1 (reference)

SD, standard deviation. 4th quartile functions as reference for quartiles 1–3. Results are presented as odds ratios with 95% confidence intervals. * Statistically significant.

patients from the top quartile had a 7.2 and 8.2 times higher risk of having CABG, respectively.

While the correlation between retinal vascular structure and CABG has not been addressed before, other researchers studied D_F and its association with cardiovascular mortality as well as risk factors [7, 8]. Lower and greater D_F were associated with higher risk of 14-year cardiovascular disease mortality in a prospective cohort study by Liew et al. [7]. Patients with D_F in the first quartile had a 44% (OR, 1.44; 95% CI: 1.13–1.83) increased risk of cardiovascular mortality, while patients in the fourth quartile had a 51% (OR, 1.52; 95% CI: 1.14–1.98) increased risk compared to patients in the second and third quartiles. Low D_F and increased risk of cardiovascular disease support the findings in our study, but cannot be directly transferred, since we only found differences for the retinal main venular D_F .

Cheung et al. [8] investigated the association between D_F and cardiovascular risk factors in a cross-sectional design showing that higher mean arterial blood pressure (coefficient -0.085 ; $p < 0.001$) and older age (coefficient -0.311 ; $p < 0.001$) were associated with lower D_F . Liew et al. [23] made a similar observation with lower D_F being associated with elevated systolic blood pressure (coefficient -0.29 ; $p < 0.0001$).

According to the *principle of minimum work* stated by Murray [24], the human circulation has an architectural optimum securing the delivery of oxygen to the tissues. D_F is a potential method to evaluate deviations from this optimal architectural structure providing new insights to circulatory diseases.

The pathophysiological mechanisms leading to sub-optimal D_F are not well understood. However, it is well known that cardiovascular risk factors such as hypertension, elevated cholesterol levels, and diabetes all dispose to atherosclerosis and microvascular alterations [25]. Antihypertensives, anticoagulants, and cholesterol-lowering drugs were all represented with statistical significance in the case group (Table 1). This indicates that cardiovascular risk factors have been present disposing to alterations in the retinal vascular complexity perhaps resulting in lower D_F . Table 1 also displays a higher representation of diabetes among the cases. Diabetic retinopathy and lower D_F were associated in the study by Grauslund et al. [26], and on the basis of this, diabetes is a potential influencing parameter in the present study.

However, these are all speculations, and the underlying mechanisms regarding lower D_F are poorly understood, and more research is needed. There is a lot of potential that retinal vascular D_F , as a noninvasive procedure, can be implemented to help clinicians evaluate and maybe even predict the risk of cardiovascular disease based on the alterations in the geometric complexity of the retinal vasculature. This involves a well-validated software application such as VAMPIRE, since the retinal vascular D_F is difficult to evaluate with the naked eye as a clinician. The difference in D_F between the lowest and highest quartiles is in particular what is clinically interesting since patients in the lowest quartile had an increased risk of having CABG. Future research should include functional measure of retinal ischemia in association with D_F to better understand the retinal pathophysiology and a larger population to better evaluate the prognostic value of D_F for CABG. Secondly, it would be interesting to investigate whether the retinal microcirculation correlates with the cardiovascular microcirculation.

Our study contains some limitations that should be mentioned. First of all, the cross-sectional design is limiting, as it does not allow elucidating causal relationships. Secondly, given the age of the study population, it was not possible to include controls without any cardiovascular risk factors, even though these were statistically significantly lower than the cases. The results are potentially influenced by the fact that cardiovascular risk factors were to some extent also present in the control group, even though these patients did not have manifest cardiovascular disease.

Refractive errors affect retinal measures in general, and Cheung et al. [8] observed an association between myopic refraction and lower retinal vascular D_F . We were not able to correct for myopia or other refractive deviations, which potentially is reflected in our overall findings. Further, we did not manage to match the groups on cardiovascular parameters, but, on the other hand, it is considered a strength that the groups are matched in age, due to research suggesting that D_F decreases with age [8, 27].

Conclusion

In summary, this study demonstrated that retinal vascular fractals, a method to assess the complexity of the retinal vascularity, associate with macrovascular cardiac disease after adjusting for gender and age. Prospective and functional studies are needed to clarify the pathophysiological mechanisms fundamental to understand the full picture. Nonetheless, we were able to shed light on the promising potential the retinal circulation has when it comes to assessing not only ocular diseases but also extraocular diseases.

Statement of Ethics

The project was approved by the regional ethics committee (project-ID: S-20170205) and the Danish Data Agency (Journal No. 18/29227). It was performed according to good clinical practice and the Declaration of Helsinki. The participants all gave their written informed consent on a voluntary and informed basis.

Conflict of Interest Statement

There are no conflicts of interest.

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Author Contributions

All authors listed have contributed to the design and manuscript. The first author did the inclusion of patients and the analysis of data.

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