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Research Article

Title: Retinal vascular fractal dimensions and their association with macrovascular cardiac disease.

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Short title: Retinal vascular fractals and CABG.

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Abstract

Introduction

As the only part of the human vasculature, retina is available for direct, non-invasive inspection. Retinal vascular fractal dimension (D_F) is a method to measure the structure of the retinal vascular tree, with higher non-integer 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 semi-automatic 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 to 31.86; p=0.009) and second quartile (OR, 8.25; 95% confidence interval, 1.70 to 40.01; p=0.009).

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 non-ocular macrovascular disease.

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 calibre (1-6), retinal vascular fractal dimension (D_F) (7, 8) and microvascular abnormalities (9, 10), suggest that retinal structural measurements can be used to detect, or even predict, cardiovascular disease.

The concept of statistical self-similarity introduced by Mandelbrot in 1967 stated that a fraction of a pattern statistically can be considered the whole (11). In nature, this can be applied to coastal lines, snowflakes, lightning as well as the retinal vascular tree (12). D_F can be measured as a non-integer between 1 and 2. The higher the number, the more 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 calibre 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 (CABG patients) compared to cardiovascular healthy controls.

Method and materials

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 coronary artery bypass grafting (CABG). Recruitment took place four days after surgery and the clinical examinations when they had pace wires and heart monitoring scope discontinued four to six 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 re-surgery 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, hypercholesterolemia, diabetes, history of smoking or if they were treated with anti-coagulants from non-cardiac causes.

Clinical characteristics were gathered from a full medical interview in addition with information from the patient records. After ten minutes of rest blood pressure was measured standardized as a mean of three measurements on the right upper arm (Omron M6 AC, Kyoto, Japan). Blood samples were collected for analysis including cholesterol profile, triglycerides and haemoglobin1Ac.

The Topcon TRC-50X (Topcon, Tokyo, Japan) was used to capture a 45° optic disc-centred 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 phenylephedrine 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 pre-specified protocol. The grader was not blinded to the group allocation of the individual participant. The method for the semi-automatic software is

outlined in detail elsewhere (19, 20) and will only be briefly explained here.
Retinal landmarks were automatically detected, including the centre and outer diameter of the optic disk and the macula centre. This enabled the establishment of a coordinate system with circular zones A (0-0.5 disc diameters from the optic disc centre), B (between 0.5-1.0) and C (between 0.5-2.0) around the optic disc (Figure 1). Vessels were automatically traced as arterioles or venules, ranging from zone A and peripheral to zone C. This creates a skeletonized pattern (Figure 2b) of the entire retinal circulation (Figure 2a). The automatic localization of the optic disc, macula and the vessels were 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 the 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, Texas, USA).
Differences between the two groups were tested by the Mann-Whitney U test used for continuous data and the Pearson chi-square 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 four quartiles.

Results

The exclusion process led to ten 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 two 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), were more likely to have diabetes mellitus (34.2% vs. 8.1%, p=0.01), but had a lower systolic (138mmHg vs. 143mmHg, p=0.01) and diastolic blood pressure (70mmHg vs. 90mmHg, p=<0.001). Cases were more commonly medicated with anti-hypertensiva (89.5% vs. 51.4%, p=<0.001), statins (94.7% vs. 29.7%, p=<0.001) and anti-coagulants (100% vs. 21.6% p=<0.001).
We found no differences between the two 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 eye of patients in the first (OR, 7.20; 95% confidence interval, 1.63 to 31.86; p=0.009) and second quartile (OR, 8.25; 95% confidence interval, 1.70 to 40.01; p=0.009). 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 to 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 two lowest quartiles compared to 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 to 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 quartile. Low D_F and increased risk of cardiovascular disease supports 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 to lower D_F. Liew et al. (23) made a similar observation with lower D_F being associated to elevated systolic blood pressure (coefficient -0.29; $p < 0.0001$).

According to *the principle of minimum work* stated by Murray in 1926 (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, diabetes all dispose to atherosclerosis and microvascular alterations (25). Anti-hypertensive, anti-coagulants 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 if 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 non-invasive 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 are 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 to 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 to 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 among 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 (8) and Cheung et al. 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 mange to match the groups on cardiovascular parameters, but, on the other hand is it considered 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, associates to 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 extra ocular diseases.

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Statement of Ethics

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

Disclosure Statement

The authors have no conflict of interest to declare.

Author contributions

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

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Figure legends

Fig. 1. A disc centered fundus photography processed for analysis of fractal dimension by 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.

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

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

	Cases (n = 38)	Controls (n = 37)	P-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, mmHg	138	143	0.01
Diastolic blood pressure, mmHg	70	90	<0.001
History of smoking (%)	71.4	78.3	0.48
Diabetes Mellitus (%)	34.2	8.1	0.01
Medication			
Anti-hypertensive (%)	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 presented as medians. Tested for differences between cases and controls. P-values <0.05 is regarded statistically significant. History of smoking = former or current, anti-hypertensive medication = all kinds of anti-hypertensive drugs, CABG = coronary artery bypass grafting, HDL-cholesterol = high-density lipoproteins-cholesterol, LDL-cholesterol = low-density lipoproteins-cholesterol, HbA1c= glycosylated haemoglobin. * = 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	N	Cardiovascular disease
Per SD decrease, 0.0555		1.83 (1.07 to 3.13)*
First quartile, 1.017 to 1.123	20	7.20 (1.63 to 31.86)*
Second quartile, 1.124 to 1.162	18	8.25 (1.70 to 40.01)*
Third quartile, 1.164 to 1.200	18	1.79 (0.39 to 8.15)
Fourth quartile, 1.201 to 1.324	18	1 (reference)

SD = standard deviation. 4. Quartile functions as reference for quartile 1-3. Results are presented as odds ratios with 95%-confidence intervals. * = statistically significant.