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Retinal microvascular structure and function in patients with risk factors of atherosclerosis and coronary artery disease

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ABSTRACT

Objective: Retinal microvascular signs are markers of cardiovascular disease risk. There are limited data, on relationships between retinal microvascular signs and retinal microvascular endothelial function. We sought to determine the relationship of retinal vascular signs with retinal microvascular endothelial function in patients with or at high risk of coronary artery disease.

Methods: Participants with atherosclerosis risk factors and coronary disease ($n = 258$; mean age 57 ± 11 years) were recruited to have static and dynamic retinal vascular assessment. Retinal arteriolar dilatation in response to flicker light (FI–RAD) was measured using the Digital Vessel Analyser and expressed as percentage increase over baseline diameter. Static retinal photographs were acquired utilising a digital fundus camera for measurement of central retinal artery and vein equivalent (CRAE and CRVE), arteriovenous nicking (AVN) and focal arteriolar narrowing (FAN).

Results: Intra-class correlation coefficient was 0.82 for flicker-light induced retinal arteriolar dilatation. There were modest associations in retinal vascular measurements between eyes. For each 10 μm decrease in retinal arteriolar diameter, the absolute increase in FI–RAD was 0.28% (95% CI 0.11, 0.45; $p = 0.002$) independent of age, gender and atherosclerosis risk factors. AVN and FAN were associated with attenuated FI–RAD ($\beta = -0.67\%$; 95% CI −1.20, −0.15; $p = 0.012$) and ($\beta = -0.83\%$; 95% CI −1.44, −0.23; $p = 0.007$) respectively after adjustment for age and gender.

Conclusion: Assessment of retinal microvascular endothelial function is reproducible and correlated with retinal microvascular structure and signs, independent of atherosclerosis risk factors. Assessment of retinal vascular structure and function may provide insights into atherosclerotic disease.

1. Introduction

The retinal circulation is unique as it allows a direct and non-invasive window to the health of the human circulation *in vivo*. Large epidemiologic studies have shown that retinal microvascular signs (e.g., arteriovenous nicking, focal arteriolar narrowing, and measurement of static retinal vascular calibre) provide information on risk of vascular complications, cardiac events and stroke in the general population [1–6].

The mechanisms and pathways underlying these associations remain, however, unclear. Advances in retinal vascular imaging technology have allowed retinal microvascular function, a nitric oxide dependent phenomenon, to be evaluated in "real time" by non-invasive dynamic assessment of retinal vascular dilatation in response to diffuse luminance flicker light using the Dynamic Vessel Analyser (DVA) [7]. There are limited data on how static retinal microvascular signs are correlated with retinal microvascular endothelial function, particularly in subjects with or at risk for coronary heart disease [8]. Understanding the associations between static and dynamic retinal vascular markers may help in further developing a diagnostic tool capable of early detection of atherosclerosis, and assist in risk stratification of patients with cardiovascular disease.

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To address these gaps, we sought to examine the feasibility of measuring retinal microvascular endothelial function using the DVA in patients with or at risk of coronary artery disease, and to study the inter-relationship between retinal vascular structure and function.

2. Materials and methods

2.1. Study population and patient preparation

The study was approved by the Human Research Ethics Committee of Austin Health and written informed consent was obtained from all subjects. All participants (n = 258) underwent retinal photography, including static and dynamic assessment. The inclusion criteria for recruitment were that participants had to have at least two traditional cardiovascular risk factors and/or clinically evident coronary artery disease. Traditional cardiovascular risk factors included a history of hypertension, dyslipidaemia, diabetes mellitus, active cigarette smoking history or a family history of premature coronary disease. Clinical evident coronary disease was defined as either a) coronary artery stenosis of >50% documented at coronary angiography, b) the presence of symptoms of myocardial ischaemia and an abnormal stress test, or c) an acute coronary syndrome. Patients were excluded if an adequate retinal examination could not be performed due to cataracts, narrow-angle glaucoma or history of epilepsy. All tests were undertaken in the morning after an overnight fast, in a quiet, temperature-controlled environment. Vasoactive drugs, caffeinated drinks and nicotine were withheld when possible for at least 12 h, to minimise the potential influence of these factors on vessel calibre.

2.2. Dynamic and static retinal vascular imaging

Retinal photographs and dynamic retinal vascular assessment were performed on both eyes in all subjects after the pupils were dilated with 1% tropicamide eye drop solution in order to obtain optimal retinal images. Assessment of flicker-light induced retinal vasodilatation was performed using the DVA (IMEDOS, Germany) to assess percentage change of retinal vessel diameter compared to baseline [9]. In brief, the patient focuses at a fixation bar positioned inside its viewing system while the fundus is examined under green light. An arteriolar and venular segment between half and two disc diameters from the margin of the optic disc is selected. The measurement starts automatically at a video frequency of 25 Hz which allows 25 readings of vessel diameter per second. The diameter of the vessel was then calculated continuously along the selected segment, which is preferably 1.5 mm in length. A baseline recording for 50 s was performed followed by a 20 s provocation by flickering light of the same wavelength at a frequency of 12.5 Hz. This was then followed by 80 s of steady illumination to allow the vessel to return to baseline. The cycle is then repeated twice with a total duration of 350 s (Fig. 1). Responses were measured in each eye and recorded for comparison. A complete examination was performed in approximately 20 min.

Static digital retinal photographs were taken of both eyes after pupil dilation (Canon EOS 40D, CF-60UVi fundus camera). A computer-based standardized protocol (IVAN, University of Wisconsin, USA) automatically measures the diameter of arterioles and venules within ½ to 1 disc diameter from the margin of the optic disc to summarize arteriolar and venular diameter [10]. The central retinal artery and vein equivalent (CRAE and CRVE) is calculated from an average of the biggest six vessels reflecting the estimated diameter of the central retinal artery and vein Ref. [11]. Other structural changes were also assessed, including focal arteriolar narrowing (FAN) and arteriovenous nicking (AVN) following the modified protocol from the Multi-Ethnic Study of Atherosclerosis [12].

2.3. Reproducibility of dynamic retinal studies

In a subset of 13 patients (38% male; mean age of 51 ± 16 years) with atherosclerosis risk factors, dynamic retinal studies were performed by the same observer and repeated after 6 weeks while medication was held constant. Intraobserver intraclass correlation coefficient (ICC) analysis was performed to assess test–retest reproducibility. ICC of 0–0.2 indicates poor agreement, 0.3–0.4 indicates fair agreement, 0.5–0.6 indicates moderate agreement, 0.7–0.8 indicates strong agreement, and >0.8 indicates almost perfect agreement [13]. Furthermore, agreement between 1st and 2nd measurements for baseline diameter and maximum dilatation of retinal arterioles and venules in the same 13 subjects was assessed, using Bland–Altman analysis [14]. A test is considered reproducible if the mean difference is close to zero and 95% of differences are less than two standard deviations.

2.4. Statistical analysis

Continuous variables were expressed as mean ± SD and categorical variables were expressed as count (%). Data were assessed for normality using the Shapiro–Wilk test and found to be normally distributed. Dynamic and static retinal vascular variables were assessed in both eyes. The relationship between the right and left eye was evaluated first and average measurements were used when appropriate to assess relationships between variables as described below. Cohen's Kappa coefficient was calculated as a statistical measure of agreement between categorical data. Pearson's correlation coefficient was used to assess associations between continuous variables. Data for FAN and AVN were dichotomized into present versus absent. Linear regression analysis was performed to assess the relationship between FI–RAD, CRAE, CRVE, FAN and AVN in each eye adjusting for cardiovascular risk factors, which were selected based on their known association with vascular function. Each outcome variable was entered separately into the multivariate analysis adjusting for age, gender, systolic blood pressure, BMI, smoking, family history of ischaemic heart disease, hypertension, dyslipidaemia, serum glucose, creatinine and cholesterol levels, statin therapy, renin–angiotensin blocker, beta-blocker and antiplatelet therapy. A p value of ≤0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 18 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Reproducibility and association between eyes

The characteristics of the study population are displayed in Table 1. ICC was 0.99 and 0.98 for baseline arteriolar and venular diameter respectively, and 0.82 and 0.79 for flicker-light induced retinal arteriolar and venular dilatation respectively. None of 13 baseline retinal arteriolar and venular diameter measurements were beyond the limits of agreement while at maximum dilatation, 1/13 of the arteriolar (FI–RAD), but not venular (FI–RVD), measurements were beyond the limits of agreement (Fig. 2). Reproducibility was similar in the left and right eyes.

The association in static and dynamic vascular measurements between the right and left eyes are displayed in Table 2. There is a modest and statistically significant association between parameters from right and left eyes (p < 0.001). Fig. 3A demonstrates the relationship between FI–RAD in the left and right eyes. Differences between eyes were similar in patients with established coronary disease or risk factors alone with correlation coefficients of 0.548 and 0.572 respectively.