CLINICAL INVESTIGATION

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Retinal vascular diameter in young subjects with a vasospastic propensity

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Abstract *Purpose:* Retinal vascular diameters have recently been shown not to be related to an increased risk of open-angle glaucoma. Because vasospastic propensity has been suggested to represent a risk factor for various ocular diseases, especially glaucoma, the steady-state retinal vascular diameter in subjects with a propensity for systemic vascular dysregulation was compared with a group of age-matched gender-matched controls. *Methods:* Thirty healthy non-smoking individuals [female/male 26/4; mean ± SD age 22.8±3.4 (range 18–31) years] were enrolled into the study. Subjects were classified as having vasospasm (15 subjects) if they related a clear history of frequently cold hands and as healthy subjects (15 subjects) if they denied such a history. Vasospastic propensity or the absence of it had to be confirmed by nail-fold capillaroscopy. Vascular diameter of retinal vessels was measured repeatedly on two days with the retinal vessel analyser and corrected for perfusion pressure, age, and refraction. *Results:* Neither retinal arteriole diameter (P=0.30) or retinal venule diameter (P=0.49), nor retinal arteriole-to-venule ratio (P=0.96), differed between the two experimental groups. *Conclusions:* Although vasospastic propensity has been suggested to represent a risk factor in various ocular diseases, the steady-state retinal vessel diameters are not altered in healthy vasospastic subjects. It is probable that the steady-state retinal vessel diameters are no adequate risk indicators for the haemodynamic risk in diseases such as glaucoma.

Keywords Glaucoma · Retina · Vascular dysregulation · Vasospasm

Introduction

Structural changes in the retinal vasculature have long been advocated to represent important predictors of systemic vascular disease [37]. However, qualitative assessment of retinal vessels, using ophthalmoscopy, has a poor reproducibility [4]. More recent studies have demonstrated that retinal microvasculature may show abnormalities related to elevated concurrent blood pressure in older people [25, 39, 42] and to cardiovascular complications [6, 26, 27, 38, 40, 41, 43, 44]. From a functional perspective, diseases with vascular dysregulation have been reported to induce vaso-spasms in the retinal vessels [2, 17, 24], and retinal blood flow abnormalities have been reported in otherwise healthy subjects with a propensity for systemic vascular dysregulation [13]. It has been suggested that such alterations may play a role in the pathogenesis of glaucoma [8, 13] and also in other ocular diseases [9]. Notwithstanding, a recent study suggested that baseline retinal vessel diameters do not predict the incidence of glaucomatous changes in the optic disc [21]. However, the latter study included only subjects aged at least 55 years, possibly excluding patients who may have developed glaucoma from vascular dysregulation at an earlier age. Therefore, the diameter of the

retinal vessels in otherwise healthy young vasospastic subjects was compared with a group of age-matched gender-matched controls in a pilot study.

Patients and methods

Thirty healthy non-smoking individuals, 26 female and four male [mean ± SD age 22.8±3.4 (range 18–31) years] were enrolled into the study. All procedures conformed to the Declaration of Helsinki. After approval by the ethics committee, we obtained informed consent from the subjects. A notification in the University Eye Clinic of Basel informed potential volunteers (collaborators, students, parents and friends of patients) of the opportunity to participate in a scientific research project. Subjects were screened for ocular and systemic diseases. A detailed medical and ophthalmic history was recorded, and all subjects completed an ophthalmological examination. Included were only individuals with no history of ocular or systemic disease, no history of chronic or current systemic or topical medication, and no history of drug or alcohol abuse. Further inclusion criteria were a normal systolic (100–140 mmHg) and diastolic (60–90 mmHg) blood pressure, a best-corrected visual acuity above 20/25 in both eyes, ametropia within −3 to +3 dioptres of spherical equivalent and less than 1 dioptre astigmatism in each eye, an intra-ocular pressure (IOP) lower than 20 mmHg in each eye by (Goldman) aplanation tonometry, and no pathological findings after slit-lamp examination and indirect funduscopy.

Subjects were classified as having vasospasm if they related a clear history of frequently cold hands (answering "yes" to the questions: "do you always have cold hands, even during the summer?" and "do other people tell you that you have cold hands?") and as healthy subjects if they reported no such history. Vasospastic propensity or the absence of it had to be confirmed by nail-fold capillaroscopy, the examiner being unaware of the history of cold hands. Subjects with contradictory findings from nail-fold capillaroscopy and history or describing "sometimes having cold hands" were excluded from the present analysis.

Nail-fold capillaroscopy

Nail-fold capillaroscopy was performed in a room with a constant temperature of approximately 23°C (range 21–25°C). Prior to the examination, the hands of the subjects were warmed in a water bath of 40°C. The skin of the nail-fold was made transparent by a drop of oil, rendering the capillaries running parallel to the skin surface and the flow of cellular elements visible under a light microscope. A microscope coupled to a TV monitor, which was coupled to a video-recorder, allowing the observed blood flow to

be videotaped and to be analysed off-line was used. After baseline flow measurement, the nail-fold area was cooled at −15°C for 60 s by rapidly decompressing carbon dioxide, and the occurrence of blood flow standstill was recorded. A closure of one or more visible capillaries with an average stop-time longer than 12 s was defined as a vasospastic reaction [29].

Retinal vascular diameter

Vascular diameter of retinal vessels was measured with the retinal vessel analyser (Imdeos, Jena, Germany), a commercially available device allowing high precision with coefficients of variation for repeated measurements below 3% [33, 35]. The retinal vessel analyser (RVA) comprises a fundus camera, a video-camera, a real-time monitor, and a personal computer with analysing software for the accurate determination of retinal arteriole and venule diameters. Retinal vessel diameters are analysed in real time with a maximum frequency of 50 Hz. For this purpose the fundus is imaged onto the charge-coupled device chip of the video-camera. The consecutive fundus images are digitised by a frame grabber. In addition, the fundus image can be inspected on the real-time monitor and, if necessary, stored on a video-recorder, allowing either on-line or off-line evaluation.

Measurement of retinal vessel diameters is based on adaptive algorithms using the specific absorbing properties of haemoglobin. A region of interest is defined by a rectangle on the screen of the real-time monitor, within which the retinal vessel diameter is calculated along the entire vascular segment. This window can include a retinal arteriole, a retinal venule, or both. The system is able automatically to correct for alterations in luminance as induced, for instance, by slight eye movements, and the system automatically stops the measurement during blinks. To correct for magnification error, we estimated the optic disc size in each subject at the slit-lamp with a Volk 60 D lens [34]. To establish the correspondence between measurements and dimensions in the eye, we assumed that the diameter of the average optic nerve head was 1,850 μm in the eye [20]. The relationship between these estimates of optic disc size was used as the basis for correcting for magnification error.

Experimental procedures

After evaluation of inclusion criteria and nail-fold capillaroscopy, two days of experiment, 1 week apart, were scheduled for each subject.

On each day of experiment, retinal vessel diameters were measured in the morning, after the subject had fasted for 12 h overnight, in one randomly chosen eye (the same eye

on both days of experiment). Participants were instructed to abstain from a large meal, alcohol consumption (including alcohol-containing products and drugs), and physical exercise for 24 h prior to the measurements. On the days of the experiments, the subjects were seated for 30 min in the laboratory, and local tropicamid was applied in one eye thrice every 5 min for pupil dilation. Blood pressure was measured every 10 min for at least 30 min. After stabilisation of blood pressure, retinal vessel diameter was assessed. The fellow eye was covered to improve fixation during imaging with the fundus camera. The image of the retina was adjusted on the screen of the real-time monitor, and a region of interest was laid over a 1–1.5 mm-long segment of the inferior major temporal branch arteriole. The measurement site was located at least half a disc diameter away from the margin of the optic disc. The average measurement time was approximately 1 min. The recordings were stored on a videotape, and the inferior major temporal branch venule was evaluated off-line at the same distance from the disc as the arteriole. Following retinal vessel assessment, blood pressure was measured by sphygmomanometry and intraocular pressure was measured by Goldmann aplanation tonometry, after the application of one drop of 0.4% benoxinate hydrochloride and the staining of the tear film with a strip of fluorescein sodium.

Ocular perfusion pressure (OPP) was calculated by the following formula: $OPP = (2/3[2/3 * DBP + 1/3 * SBP]) - IOP$ where DBP, SBP, and IOP represent diastolic blood pressure, systolic blood pressure, and intraocular pressure, respectively.

Particular emphasis was put on ensuring the same measurement conditions at the two sessions, especially for the adjustment of brightness control. All measurements executed with the RVA were performed by the same experienced researcher, who, as the investigator doing nail-fold capillaroscopy, was blind to the vasospastic propensity of the subjects. For the second measurements, the investigator was blind to the selection of the region of interest in the first measurement. We obtained the second measurements in order to account for the variation of the placement of the measurement window between participants, as well as for physiological variation of retinal vessel diameter. In order to ascertain that repeated measurements were obtained at comparable conditions, we excluded subjects showing a change of more than 8% between the first and the second measurements.

Although this was a pilot study, sample size calculations were applied. The variability for measurements of the arteriolar diameter was considered for power calculations, because its measurement is prone to a higher variability [33, 35]. Based on four unpublished series of measurements, the average within-group variability was estimated to be 12% of the mean. For a statistical power of 80% and a probability of 5%, to find a difference between groups of 13% (slightly higher than the within-group variability), we needed to recruit 14 subjects for each group.

We performed a two-way analysis of variance (vasospastic subjects/non-vasospastic subjects × measurement 1/measurement 2), with the two sessions set as repeated measurements, to compare retina arteriolar and venular vessel diameters, as well as the arteriole-to-venule ratio (arteriole diameter/venule diameter) between vasospastic subjects and controls, after correcting the measurements for perfusion pressure, age, and refraction. A P value of 0.05 was considered significant.

Results

Ten subjects were excluded because of inconsistent findings between history and nail-fold capillaroscopy, five because of bad recordings in nail-fold capillaroscopy, seven because of bad recordings, and three because of a high differences in RVA measurements between the two days of experiment. Recruitment was pursued until 15 vasospastic and 15 age-matched controls had been identified. Age was 23.0±3.3 years in vasospastic subjects and 22.7±3.6 years in controls (P=0.78). Spherical equivalent for refraction was −0.50±1.28 dioptres in vasospastic subjects and −0.79±1.19 dioptres in controls (P=0.53).

IOP, SBP, DBP and OPP are detailed in Table 1 for both measurement sessions. None of the haemodynamic parameters, evaluated in a two-way analysis of variance model (vasospastic subjects/non-vasospastic subjects × measurement 1/measurement 2) with the two sessions set as repeated measurements, showed a difference between the two experimental groups (P=0.96, P=0.65, and P=0.82 for SBP, DBP and OPP, respectively). There was no statistical difference between the two sessions (P=0.19, P=0.21, and P=0.30, respectively), and the two experimental groups were comparable in this regard (interaction between experimental groups and repeated measurements: Pv=0.77, P=0.58, and P=0.51, respectively). IOP in contrast, was

Table 1 Intraocular pressure, systolic blood pressure, diastolic blood pressure, and perfusion pressure at both measurement sessions (mean ± SD)

Parameter	Session	Vasospastic subjects	Controls
Intraocular pressure	1	12.2+1.5	13.6+1.9
(mmHg)	2	12.1+1.5	13.3+1.7
Systolic blood pressure	1	115.8+13.4	115.2+12.3
(mmHg)	2	113.7+14.7	113.8+12.56
Diastolic blood pressure	1	74.1+10.4	75.2+9.7
	2	72.3+10.7	74.5+9.7
Perfusion pressure	1	46.5±6.9	45.4±7.0
(mmHg)	2	45.3±7.2	45.2±6.8

Table 2 Diameters of retinal arterioles and venules, and arteriole-to-venule ratio at both measurement sessions (mean ± SD)

Parameter	Session	Vasospastic subjects	Controls
Arteriolar diameters (μm)	1	126.8±14.7	121.2±15.5
	2	127.6±16.1	121.3±15.8
Venular diameters (μm)	1	154.7±16.4	150.3±22.8
	2	155.1±15.4	149.7±21.3
Arteriolar-to-venular ratio	1	0.83±0.09	0.82±0.1
	2	0.83±0.1	0.83±0.1

lower in vasospastic subjects (P=0.04), with no statistical difference between the two sessions (P=0.16) and no statistical significant interaction between experimental groups and repeated measurements (P=0.35).

The measurements for the diameters for the arterioles, the venules, and the arteriole-to-venule ratio were corrected for the potential influence of age, perfusion pressure and refraction. The values are detailed in Table 2 for both measurement sessions. Evaluation of the corrected values in a two-way analysis of variance model (vasospastic subjects/non-vasospastic subjects × measurement 1/measurement 2), with the two sessions set as repeated measurements, showed no difference between the groups (P=0.30), and there was no statistical difference between the two sessions (P=0.28), the two experimental groups behaving similarly in this regard (interaction between experimental groups and repeated measurements P=0.48). Retinal venule diameter, evaluated in a similar model, showed no difference between the groups (P=0.49) or between the two sessions (P=0.94), and there was no interaction between the experimental groups and the repeated measurements (P=0.54). Similarly, retinal arteriole-to-venule ratio showed no difference between the groups (P=0.96) or between the two sessions (P=0.70), and there was no interaction between the experimental groups and the repeated measurements (P=0.99). A comparison of the diameters for the arterioles, the venules, and the arteriole-to-venule ratio, without correction for the potential influence of age, ocular perfusion pressure and refraction, did not alter these findings.

Discussion

The present study demonstrated that retinal vascular diameter is not altered in young and otherwise healthy vasospastic subjects. All the vascular measurements were compared after correction for ocular perfusion pressure, age or refraction, but, except for a lower IOP in vasospastic subjects, all factors potentially having some bearing on the retinal vascular diameter, especially ocular perfusion pressure, were comparable between the experimental groups.

The vasospastic syndrome is a condition manifesting as reversible, local or generalised arteriolar constrictions (vasospasms) or inadequate dilation [9], possibly due to a vascular endotheliopathy [19, 28, 32] and often associated with vasospastic reactions to cold in the nail-fold capillaries, as assessed by nail-fold capillaroscopy [29]. The primary vasospastic syndrome is encountered more often in women than in men, and is characterised by a tendency towards cold hands. The vascular attacks can be provoked by many factors, such as exposure to cold, stress, emotions and nicotine. The observation of parallel changes in peripheral blood flow (fingers) and visual field in vasospastic patients [16] led to the definition of an entity called "presumed ocular vasospastic syndrome" [7]. Additional studies led further support to the idea that some parallelisms in ocular and digital blood flow exist [11, 15] and that the presumed ocular vasospastic syndrome is associated with a lack of blood flow autoregulation [12, 14, 18]. Ocular vasospastic phenomena have also been observed directly in patients with unstable primary angina [2] and with migraine [24]. Ocular vasospastic phenomena have been advocated to be a potential risk factor in various ocular conditions, including glaucoma [1], retinal venous occlusion [30], acute ischaemic optic neuropathy [23], and central serous chorioretinopathy [36].

The retinal vessel diameter was assessed with the RVA, a device allowing a high precision for repeated measurements [33, 35] and for which a good ability to detect changes after interventions has been demonstrated [10, 22, 31]. Furthermore, Dorner et al. [5] calculated the diameter of the central retinal artery based on retinal blood flow velocity measurements obtained by laser Doppler velocimetry and colour Doppler imaging as well as retinal venous diameter measurements with the RVA. Their results were in good agreement to in vitro measurements, suggesting a good ability for the RVA to estimate the retinal vascular diameter. Although vessel diameter assessments were based on measurements obtained in 1 min, which prevented vascular pulsation from having a relevant influence on the results, other physiological parameters, for example hormonal effects [3], as well as variability in alignment of the measurement window were likely to influence the measurements and have a relevant bearing on a small sample. For this reason, repeated measurements were obtained on two different days, 1 week apart, and only repeated measurements with a fair reproducibility were considered in the present study. Tropicamid was used to dilate the pupils and, although this substance was not expected to have a vascular effect, one may have to consider a possible effect of this drug on vessel diameter. However, because it was used equally in both groups, it seems unlikely that this substance may have influenced the presented results. A limitation in our study is the fact that only one pair of vessels was assessed within a defined distance from the optic disc. It is thinkable that vessels of a

smaller calibre, or those in the upper retina, may be more prone to disclose differences between the analysed subjects. Further investigations are needed to evaluate the regional importance of alterations in the calibre of retinal vessels.

The current study suggests that, under steady-state conditions, the retinal vasculature of otherwise healthy vasospastic subjects does not illustrate distinctive features in retinal vascular diameter. A recently published prospective study showed that both retinal arteriole and venule diameters are not related to an increased risk of open-angle glaucoma [21]. Because vasospastic propensity has been suggested to represent a risk factor for various ocular diseases, especially glaucoma [9], the present findings suggest that the assessment of the retinal vascular diameter may not be an adequate approach to assess the haemodynamic risk in glaucoma patients.

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