MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2014; 20: 2607-2616
DOI: 10.12659/MSM.891069

Received: 2014.05.21
Accepted: 2014.08.25
Published: 2014.12.11

Prolonged Postocclusive Hyperemia Response in Patients with Normal-Tension Glaucoma

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Source of support: We received a grant from the Polish Ministry of Science and Higher Education (Nr N N402 165637)

Background: It is believed that endothelial dysfunction may be a link between systemic and ocular dysregulation in glaucoma. The aim of this study was to evaluate peripheral vascular reactive hyperemia in response to occlusion test and to correlate peripheral vascular findings with retrobulbar hemodynamics parameters in patients with normal-tension glaucoma.

Material/Methods: Forty-eight patients with normal-tension glaucoma (mean age 58.1 years, 38 women) and 40 control subjects (mean age 54.1 years, 36 women) were subjected to a brachial arterial occlusion test and color Doppler imaging (LOGIQ 9, GE Medical Systems) of the retrobulbar arteries. Finger hyperemia was assessed by using a 2-channel laser Doppler flowmeter (MBF-3D, Moor Instruments, Ltd.). Time parameters (time to peak flow, half-time of hyperemia, time of recovery) and amplitude parameters (maximum hyperemia response, biological zero) of the post-occlusive reactive hyperemia signal pattern as well as velocities and resistance index of the ophthalmic, central retinal, and short posterior ciliary arteries were evaluated and compared between study groups.

Results: In glaucoma patients, time to peak flow and half-time of hyperemia were significantly longer (21.4 *vs.* 12.0 s, p=0.02 and 74.1 *vs.* 44.2 s, p=0.03, respectively) and biological zero was significantly lower (2.4 *vs.* 3.2, p=0.01) comparing with healthy subjects. In glaucoma patients, peak-systolic and end-diastolic velocities of central retinal artery were significantly lower (12.8 *vs.*14.1, p=0.03 and 3.9 *vs.* 4.7, p=0.01, respectively) and resistance index of this artery was significantly higher (0.69 *vs.* 0.67, p=0.03) compared to controls. In the glaucoma group, maximum hyperemic response was negatively correlated with the resistance index of temporal short posterior ciliary arteries (r=–0.4, p=0.01), whereas in the control group half-time of hyperemia was negatively correlated with end-diastolic velocity of the central retinal artery (r=–0.3, p=0.03).

Conclusions: Arterial occlusion test elicited a prolonged systemic hyperemia response in patients with glaucoma as compared with healthy subjects. Retrobulbar blood flow alterations in glaucoma patients may be related to systemic vascular dysregulation.

MeSH Keywords: Glaucoma, Open-Angle • Hyperemia • Laser-Doppler Flowmetry • Microvessels

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/891069

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Background

The pathogenesis of glaucomatous optic neuropathy can involve both mechanical and vascular factors, either independently or by influencing one another. Evidence has accumulated to suggest that vascular insufficiency in the optic nerve head (ONH) capillaries plays an important role on the pathogenesis of normal-tension glaucoma (NTG). An alteration in the quality of blood supply at the ONH could be triggered by increased intraocular pressure (IOP), defective cardiovascular neuroregulation, abnormal blood pressure (BP), local vasospasm, autoregulatory defects, altered rheological characteristics of the blood, structural abnormalities of the vasculature, or abnormal translaminar pressure [1–8].

It is believed that in patients with NTG the mechanisms of autoregulation are disabled and unable to respond effectively to a transient and repeatable reduction of the ocular blood flow (OBF). Low BP and primary vascular dysregulation (PVD) have been suggested to be the strongest contributing factors leading to oligemia/hypoxia in glaucoma and sensitizing the optic nerve to hypoxia/reperfusion damage. PVD is associated with the imbalance of vasoactive factors produced by the vascular endothelium [9]. Endothelium produces both the strongly vasodilators such as nitric oxide (NO), prostacyclin, endothelium-derived relaxing factor (EDRF), natriuretic factor, and acetylcholine, as well as vasoconstrictor substances such as endothelin-1 (ET-1), angiotensin II, and thromboxane A_2. NO is an important regulator of blood flow, and many vasoconstrictors modulate their own action by release of NO and EDRF from the endothelium, which restores the vascular tone balance [10]. NO inhibits both platelet aggregation and the release of vasoconstrictor substances, and thus protects endothelial cells from apoptosis.

Endothelial dysfunction (ED) is a systemic disturbance consisting of a triad of symptoms: attenuated endothelium-dependent vasodilation, augmented vasoconstriction, and structural remodeling of microvessels. ED contributes to irreversible damage to blood vessels by stimulating atherosclerosis, the induction of inflammation, and proliferation of vascular wall. The process that influences endothelial damage is oxidative stress (OS), associated with oxygen free radicals formation, lipid peroxidation, inactivation of NO and NO synthase (NOS), and thus with an increased release of ET-1 [11]. Biological effects of ET-1 in the eye are: arterial vasoconstriction, depolarization of astrocytes (possibly leading to spreading depression of the neural tissue and increase glucose consumption) [12], interruption of retrograde axonal transport [13], and finally the activation of metalloproteinases (MMP-2), tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2) and other toxins (NO, tumor necrosis factor α, transforming growth factor β, glutamate) involved in remodeling of extracellular matrix within the lamina cribrosa [14].

Patients with newly diagnosed primary open-angle glaucoma (POAG) have a significantly reduced antioxidant capacity, mainly due to abnormal NO homeostasis. In patients with POAG, reduced plasma levels of glutathione, abnormal level of endothelial activation markers (VEGF, plasminogen activator-1, soluble thrombomodulin, von Willebrand factor) [15], and reduced endothelial progenitor cells have been found [16]. The imbalance between NO and ET-1 in the eye circulation results in greater susceptibility of the optic nerve to damage caused by both compression under statistically "normal" IOP, and by transient and short-term changes in perfusion pressure, caused by physiological (or therapeutic) lowering of BP, or emotional or physical vasospastic stimuli [17].

A clinical manifestation of ED is PVD [18]. People with PVD have a congenital tendency to a different vascular response to various stimuli such as cold and mechanical or emotional stress. These reactions can be studied by means of capillaroscopy, near-infrared spectroscopy, brachial artery ultrasound assessment of endothelium-dependent flow-mediated vasodilation (FMD), and laser Doppler flowmetry (LDF). To increase the reliability of the latter methods, sympathetic provocation tests such as the hand-grip test and occlusion test are used. To date, there are no published reports on the course of post-occlusive hyperemia response (POHR) in patients with glaucoma as measured by LDF.

It has been proposed that NTG may be a component of generalized vascular abnormalities that produce alterations in both the ocular and systemic circulation. Blood flow is particularly unstable in ONH. Because blood flow in the ONH is mainly regulated by the vascular endothelium and no efficient blood brain barrier exists in the ONH, circulating molecules such as vasoactive hormones or enzymes have direct access to the smooth muscle cells and pericytes of the vessels in the ONH. It was shown in *in vitro* studies that in the human ophthalmic artery (OA), endothelium-derived NO and ET-1 are very potent modulators of vascular tone [19]. A study by Gherghel [20] also demonstrated altered vascular regulation in the retinal circulation of subjects with vasospasm.

The aim of the present study was to evaluate the characteristics of the peripheral microvascular system through the analysis of the POHR within the distal part of the left upper limb and to correlate peripheral vascular findings with the retrobulbar circulation parameters as measured by color Doppler imaging (CDI) in patients with NTG and in healthy volunteers.

Material and Methods

The study was performed according to the tenets of the Declaration of Helsinki and was approved by an institutional

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Table 1. Study group characteristics.

	Glaucoma group	Control group	p-value
Age (years) (SD)	58.1±10.46	54.1±12.8	0.107*
Gender (F/M)	38/10	36/4	0.069**
SBP (mmHg) (SD)	124.8±10.0	126.1±10.3	0.745*
DBP (mmHg) (SD)	75.7±9.0	77.4±7.3	0.717*
Smokers	13	11	0.864**
BCVA	0.92±0.15	0.95±0.1	0.688***
IOP (mmHg) (SD)	13.3±2.2	12.7±3.0	0.554*
CCT (nm) ±SD	530.6±34.6	547.7±33.3	0.016*
MD (dB) (SD)	–5.07±3.48	–0.57±0.76	0.000*
PSD (dB) (SD)	4.87±2.94	1.42±0.38	0.000*

SD – standard deviation; F – female; M – men; SBP – systolic blood pressure; DBP – diastolic blood pressure; BCVA – best-corrected visual acuity; IOP – intraocular pressure; CCT – central corneal thickness; nm, nanometers; MD – mean defect; dB – decibels; PSD – pattern standard deviation; * t-Student test,** Chi^2-test, *** U Mann Whitney test.

review board at the Military Institute of Medicine, Warsaw, Poland. Signed informed consent was obtained from all patients and control subjects before study enrollment.

Patients of either sex, aged ≥18 with previously untreated or treated, early-to-moderate NTG and healthy volunteers, matched for sex and age, were enrolled. NTG was defined as glaucomatous optic nerve neuropathy characterized by IOP level ≤21 mmHg, cup-to-disc ratio greater than 0.6 or an interocular cup-to-disc ratio asymmetry greater than or equal to 0.2, and at least 1 of the following abnormalities: thinning of the rim, notching, nerve fiber layer defects, or peripapillary atrophy, with repeatable reliable repeatable glaucomatous visual field defects demonstrated in the central 24-2 program of Humphrey threshold perimetry. Early and middle-stage glaucoma were defined on the basis of the Mean Defect (MD) index of visual fields less than –6 decibels (dB) and between –12 dB and -6 dB, respectively, and on the basis of a vertical cup/disc ratio less than 0.8.

Exclusion criteria were: ocular hypertension other than NTG types of glaucoma, history of eye surgery, trauma and inflammation, myopia above – 6.0 diopters, corneal dystrophies, any significant cardiovascular, and pulmonary and metabolic conditions other than controlled arterial hypertension (HT). The control group comprised subjects without glaucoma, recruited from patients' family members and other volunteers. Previously diagnosed glaucoma patients were instructed to stop using antiglaucoma medications 4 weeks before examination. Systemic medications were not discontinued.

The study included 88 people – 48 NTG patients and 40 healthy subjects. The characteristics of the study groups are presented in Table 1.

All subjects underwent an eye examination that included: medical history, best corrected visual acuity (BCVA), slit-lamp and stereo optic disc evaluation, Goldmann applanation tonometry, central corneal thickness (CCT) measurement using ultrasonic pachymetry (OcuScan RxP, Alcon), and Humphrey central 24-2 threshold perimetry test. Ocular hemodynamics were assessed by CDI, using LOGIQ 9 CDI System (General Electric Medical Systems, Milwaukee, Wisconsin, USA) with a 6-15 MHz linear probe and was carried out in the morning hours by the same investigator (JW). Peak systolic (PSV) and end-diastolic velocities (EDV) were measured in the ophthalmic (OA), central retinal arteries (CRA), and temporal and nasal short posterior ciliary arteries (TSPCA, NSPCA), and Pourcelot's resistance index (RI) was calculated for each vessel. Flow velocity in the OA was measured close to its crossing to the optic nerve and the angle between transducer and the orientation of the vessel was corrected. CRA was localized along its course through the optic nerve and measurement was performed immediately behind the globe.

A 2-channel laser Doppler instrument MBF-3D (Moor Instruments, Devon, UK) was used for the blood perfusion measurements in the microvascular net. The measurement device is described in detail elsewhere [21]. Two optical fiber probes – basic and integrating – were used to measure perfusion in different tissue volumes. In the basic probe, separation between emission and detection fibers was 0.3 mm, whereas in the integrating probe, 8 optical fiber detectors were located around the emission fiber at 1 mm distance.

To provide reproducibility of the measurements results, the LD instrument was calibrated before each measurement. Blood perfusion measurements were carried out in air-conditioned room

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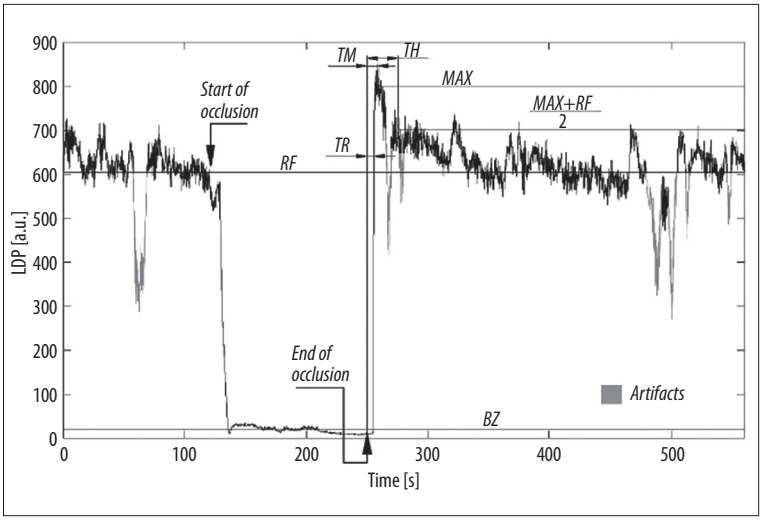

Figure 1. Flux record during occlusion test. LDP – laser Doppler perfusion; a.u. – arbitrary units; RF – resting flow; TR – time of recovery; TM – time to peak flow; TH – half-time of hyperemia; MAX – maximum hyperemia response; BZ – biological zero; s – seconds.

Table 2. Parameters of hyperemia response.

RF	Rest flow; the mean flux signal during the pre-occlusion period; presented in arbitrary units
BZ	Biological zero; the mean remaining flux signal during occlusion; expressed in percentage of RF value
MAX	Maximum hyperemia response; the highest flux signal of post-occlusive hyperemia response; expressed in the percentage increase of the perfusion signal in the relation to the RF value
TR	Time of recovery; time from the end of occlusion until the moment when flux signal returns to the rest values; presented in seconds
TM	Time to peak flow; time from the moment of the end of occlusion until the moment of maximum hyperemic response; presented in seconds
TH	Half-time of hyperemia; time from the moment of the end of occlusion until the moment when flux signal reaching the maximum value will fall by half; presented in seconds

RF – rest flux; BZ – biological zero; MAX – maximum hyperemia response; TR – time of recovery; TM – time to peak flux; TH – half-time of hyperemia.

at 22±1°C, between 8 a.m. and 10 a.m. by the same investigator (AZ) masked to the individual characteristics of the study patients. The examined subject did not take any medications, did not drink coffee, and did not smoke cigarettes at least 6 hours before the examination. The measurement procedure was carried out in a sitting position after about 20 min of rest. Before perfusion measurement, the arterial blood pressure was measured. The optical probes were attached with double-side adhesive discs to the middle fingertip (basic probe) and to the index fingertip (integrating probe) of the left hand and the patient rested the arm on a table. The post-occlusive hyperemia test included the following perfusion measurement steps: (i) 5 min of rest, (ii) 2 min of arterial occlusion on the left arm using a 15-cm – wide inflated cuff placed above the elbow, and (iii) 15 min after releasing the cuff pressure (post-occlusive hyperemia). The pressure in the cuff was set at 50 mmHg higher than the systolic BP measured on the left arm before the procedure. Amplitude and time parameters of measured perfusion signals LDF (Laser Doppler Flux) are presented in Figure 1 and listed in Table 2.

Perfusion signals were collected with 5-Hz sampling frequency through a serial interface RS232 and stored on a PC computer disk. Post-occlusive hyperemia parameters of measured perfusion signals were calculated with software developed in the Matlab environment. Value of the rest flow (RF) was calculated according to the algorithm based on a histogram of perfusion values. RF value was an arithmetic mean of first moment and the most frequent value of the histogram. The histogram was built from 1/3 perfusion signal before start of the occlusion and filtered with a zero-phase shift filter. The same algorithm was applied to estimate the biological zero (BZ) value. The derivative of the perfusion signal was calculated and the starting moment of the occlusion was obtained when the decrease of the derivative was noted. Maximum of the hyperemia (MAX) was assessed from first 4 min of the perfusion signal after the end of the occlusion. Time parameters of the hyperemia signal TR, TM, and TH were calculated taking into account the perfusion signal sampling frequency and are presented in seconds. The value of BZ was expressed in percentage of RF

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Table 3. Hyperemia response parameters of study groups.

Parameter			Mean	SD	p-value
TR (s)	TR*	NTG	6.48	3.76	0.32***
		Control	5.64	5.03	
	TR**	NTG	6.68	4.54	0.36***
		Control	5.62	4.28	
TM (s)	TM*	NTG	14.87	13.18	0.20***
		Control	10.51	7.76	
	TM**	NTG	21.43	18.01	0.02***
		Control	11.99	8.53	
TH (s)	TH*	NTG	59.36	102.06	0.57***
		Control	57.43	89.17	
	TH**	NTG	74.10	73.98	0.03***
		Control	44.18	39.00	
BZ (%)	BZ*	NTG	2.89	1.16	0.53***
		Control	3.14	2.11	
	BZ**	NTG	2.29	0.87	0.01***
		Control	3.13	1.62	
MAX (%)	MAX*	NTG	48.08	63.15	0.34***
		Control	71.47	166.63	
	MAX**	NTG	48.43	97.43	0.69***
		Control	87.12	169.93	

SD – standard deviation; TR – time of recovery; s – seconds; TM – time to peak flow; TH – half-time of hyperemia; BZ – biological zero; MAX – maximum hyperemia response; * values of basic probe; ** values of integrating probe; *** U Mann-Whitney test.

value. The MAX value was the percentage increase of the perfusion signal in the relation to the RF value.

Parameters BZ, MAX, TR, TM, and TH were assessed from measured data obtained from 2 optical fiber probes. The parameters were compared between study groups.

All statistical analysis was performed using SAS version 9.2. For continuous variables, mean values and standard deviation were determined. To compare the variables between both groups, non-parametric Mann-Whitney U test was used. To demonstrate the differences in the distribution of variables in both groups, the t-test, chi-square, and Mann-Whitney U test were used. Multiple regression analysis was used to examine the relation between hyperemia parameters as the outcomes of interest and age, sex, and HT as covariates. The correlations between the parameters in each group were tested using Pearson's r correlation coefficients. The differences were considered statistically significant at the level of $p<0.05$.

Results

No statistical differences were noted between study groups with regards to age, sex, BCVA, or IOP. There were significant differences in visual field parameters (mean defect, MD and pattern standard deviation, PSD) and CCT between NTG patients and control subjects (Table 1).

In terms of some POHR parameters, significant differences between patients with NTG and control subjects were found. In NTG patients, TM and TH were significantly longer (21.4 *vs.* 12.0 s, p=0.02 and 74.1 *vs.* 44.2 s, p=0.03, respectively, for the integrating probe) as compared to the control group. The TM and TH for the

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Table 4. CDI hemodynamic parameters.

	Glaucoma group	Control group	p-value
OA PSV	32.5±5.9	34.5±6.9	0.16*
OA EDV	8.5±3.3	8.8±3.0	0.45*
OA RI	0.75±0.06	0.75±0.06	0.86*
CRA PSV	12.8±1.9	14.1±3.1	0.03*
CRA EDV	3.9±0.8	4.7±1.3	0.01*
CRA RI	0.69±0.05	0.67±0.06	0.03*
TSPCA PSV	7.4±1.0	7.6±1.0	0.42*
TSPCA EDV	2.7±0.6	2.9±0.7	0.38*
TSPCA RI	0.63±0.07	0.62±0.07	0.78*
NSPCA PSV	7.5±1.0	7.6±1.1	0.60*
NSPCA EDV	2.8±0.6	2.9±0.6	0.51*
NSPCA RI	0.63±0.06	0.62±0.06	0.25*

OA – ophthalmic artery; CRA – central retinal artery; TSPCA – temporal short posterior ciliary artery; NSPCA – nasal short posterior ciliary artery; PSV – peak systolic velocity; EDV – end diastolic velocity; RI – resistance index; * U-Mann-Whitney test.

basic probe were also longer in patients with NTG than in healthy subjects, but the differences were not statistically significant.

The minimum flow under occlusion, biological zero (BZ), was significantly lower in patients with glaucoma than in healthy volunteers for the integrating probe (2.4 *vs.* 3.2, p=0.01). There was no significant difference in TR and in the maximum amplitude of hyperemia response (MAX) between study groups, although MAX values were lower in NTG patients than in control subjects. The characteristics of post-occlusive hyperemia signals in both groups are presented in Table 3.

In NTG patients, the CRA PSV and CRA EDV were significantly lower and the CRA RI was significantly higher as compared to the control group. There were no statistically significant differences in the OA and NSPCA velocity indices or RI in patients with NPG compared with those of normal subjects (Table 4). In NTG patients, maximum hyperemic response for both probes were correlated negatively with TSPCA RI (r=–0.4 p=0.01 and r=–0.4, p=0.01, respectively). In the control group, TH for the integrating probe was negatively correlated with CRA EDV (r=–0.3, p=0.03). Multivariate analysis showed a significant correlation between TM for both probes and NTG (t=2.35 p=0.02 and t=3.95 p=0.00). There was also a relation noted between TH for integrating probe and age, sex, and arterial hypertension.

Discussion

To the best of our knowledge, this study is the first to measure skin microcirculation by using laser-Doppler flowmetry and a brachial artery occlusion test in patients with NTG.

Data from the literature indicate that the incidence of ED is correlated with the occurrence of POAG [22]. Clinical and experimental myographic [23], plethysmography [24], and ultrasound studies [25] have provided evidence that patients with NTG present generalized ED.

For several decades, the skin vascular bed has been an a convenient model for the study of *in vivo* disturbances of microcirculation in systemic vascular diseases such as hypertension, coronary heart disease, and diabetes [26]. Fingertip skin consists of non-vascular and transparent epidermis (0.4 mm thick) and lying beneath the dermis (0.8–1.4 mm thick). Both layers are connected by dermal papilla, in which wavy loops of capillaries with a length of 0.2–0.4 mm and a diameter of 10 μ penetrate. These capillaries extend from the subpapillary plexus and run vertically toward the surface of the skin. In the subpapillary layer, superficial veins and arteries run parallel and form 2 vascular networks – a subpapillary plexus (with a diameter of 40–60 μ) and a deeper lying superficial arterial network (with a diameter of 100 μ). Blood flow through this system is regulated mainly by arterio-venous anastomoses (AVA), which are innervated by sympathetic postganglionic fibers. Flow is also regulated by precapillary sphincters and by spontaneous contraction and relaxation of the muscular component of the vascular wall (vasomotion) [27].

The laser Doppler technique allows for non-invasive and continuous measurements of skin blood perfusion in real time. A good correlation between results of perfusion measurements carried out with utilization of laser Doppler technique and clearance of xenon (Xe-133) shows credibility of the optical method [27]. The 2 optical fiber probes used in our study allowed

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us to assess the blood perfusion signal with depth discrimination [28]. Light detected by the basic optical probe comes, with high probability, only from the superficial capillary net layer, while the light detected by the integrating probe originates also from the deeper layer containing arterioles, veins, and AVA [21].

The occlusion test is designated as an endothelium-dependent process that reflects the relaxation of brachial artery when exposed to increased flow and shear stress (SS) following arterial occlusion. The main SS vector is directed perpendicular to the long axis of the vessel and causes the mechanical irritation of endothelium, which subsequently modifies its secretory activity. The endothelial signaling cascade leading to the conversion of mechanical signals into the release of vasodilatory substances has not been fully defined.

Myogenic, neural, and local factors, such as potassium ions, hydrogen ions, carbon dioxide, catecholamines, prostaglandins, and adenosine, are believed to contribute to vascular smooth muscle relaxation. The most important factor that plays a key role in modulation of POHR is endothelial NO (eNO) [29,30]. It is also believed that other endothelium-derived agents, such as prostaglandins and endothelium-derived hyperpolarizing factors (EDHFs), are involved in the mechanism of POHR. EDHFs move from endothelium to vascular smooth muscle cells via myoendothelial gap junctions, resulting in relaxation of the vessel [31]. It is postulated that vasoactive substances from the sensory nerves might play a role in reactive hyperemia in the cutaneous circulation. Although it has been shown that adrenergic vasoconstrictor nerves are not involved in the POHR, the role of cholinergic fibers has not yet been determined [32].

In our study, post-occlusive reactive hyperemia measured in patients with the NTG revealed vascular endothelial dysfunctions. Some parameters, like time to the maximum of hyperemia (TM) and half-time of hyperemia (TH), were significantly longer in patients with NTG than in healthy subjects. Dakak et al. [29] showed the influence of eNO on all phases of hyperemia reaction. Prolongation of the hyperemic reaction and decrease of its amplitude was observed in patients with insulin-dependent diabetes [33] and peripheral occlusive arterial disease [34]. We also observed lower values of the minimum flow during the occlusion (biological zero BZ) in patients with NTG as compared to controls. This difference was visible only in signals measured with the integration probe. Other authors, utilizing thermal tests, recorded different perfusion signals from optical probes with different distances between emitting and detecting optical fibers [35]. They observed decrease of the perfusion signal obtained from the probe with larger optical fibers separation (0.7 mm), whereas no change in perfusion signals was observed from the probe with shorter optical fibers separation (0.3 mm). Skin compartment containing arterioles and veins has higher flow volume than superficial areas supplied mainly by the capillaries with low flow velocity.

Several parameters can be quantified from the flux response [33,34,36]. However, no consensus exists concerning amplitude flux response parameter selection. In clinical studies, the response was expressed as maximum hyperemia flow [37], raw value of the peak minus baseline and area under the curve (AUC) [38] or raw value of the peak minus biological zero [39], percentage of baseline value [40], and increase in post-ischemic flow using the AUC at baseline and post-occlusion, as well as by the analysis of the time-flow curves during reactive hyperemia [41]. It has been reported that the expression of data in terms of AUC provides more inter- and intra-subject variability than does the expression of data standardized to a maximum vasodilatation [42]. Data expressed as absolute values seem to have lower variability than the data expressed as a percentage of baseline [39]. Some studies suggest that the maximum hyperemic flow (MAX) may indirectly reflect the ability of vascular endothelium to produce NO [29]. It was reported that inhibition of NO production by N^G-monomethyl-L-arginine (L-NMMA), an NOS inhibitor infusion, significantly decreases MAX as well as the total hyperemic flow in flow-time curves by 30–50% [43]. In our study, the MAX value was higher in the group of healthy subjects, but with no significantly difference between study groups. Other authors have also noted lack of statistically significant differences in this parameter between the patients with chronic peripheral occlusive arterial disease and healthy subjects [44].

BZ may also serve as an important parameter in some clinical situations [21]. Perfusion signal during occlusion is never equal to zero because of chaotic blood cells movements (caused by the Brownian motion). Non-zero BZ value is also an effect of spontaneous activity of the microvascular net in response to the stopped blood flow [45]. In the present study, higher value of the BZ in the signals from the integrating probe than from the base probe was observed. Increase of the BZ value with increase of the separation between emitting and detecting optical fibers and consequently with the increase of measured tissue volume was also reported by others [21]. Among time-parameters, half-time of hyperemia (TH) seems to be a more useful indirect index of endothelial function than time to peak flow (TM) because there are reports suggesting that NO may significantly contribute to the late phase of reactive hyperemia [46]. Jarm [34] showed that the dynamics of response expressed by time parameters were far better indicators of peripheral vascular disorder than the amplitude of hyperemic response measured by LDF (MAX) as well as by near-infrared spectroscopy (NIRS) or the rest of the partial pressure of oxygen (pO_2) value measured by transcutaneous oximetry ($TcPO_2$).

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Several mechanisms may contribute to reduced post-occlusive vasodilation in patients with glaucoma: decreased NOS activity and/or reduced bioavailability of endogenous NO due to deficiency of NOS, L-arginine/NOS system dysfunction [47], reduction of cGMP plasma levels [48], increased levels of endogenous NOS inhibitors, NOS-containing cell loss by apoptosis [49], and a very rapid metabolism of NO as a result of the interaction of NO with peroxides. Excessive activity of endothelin receptor A (ETA) [50], a selective defect in endothelin receptor B (ETB) and serotonin (5-HT) [51], and abnormal activity of endothelium-derived hyperpolarizing factor (EDHF) [52] may underlie the abnormal course of hyperemic response.

No satisfactory methods exist to measure alteration in the quality of blood supply at the ONH of glaucoma patients. The present study showed significantly lower values for both velocity indices of CRA in NTG patients compared with healthy subjects, which is in accordance with other authors' findings. It has been demonstrated in an *in vitro* model that the parallel changes in systolic and diastolic velocities might indicate the volumetric change of the blood vessel. Although CDI is unable to measure blood vessel diameter and blood flow, decreased PSV and EDV of such small vessel like CRA at rest may be an indirect parameter of reduced flow as a result of concurrent vasospasm [53]. In patients with glaucoma, reduction of PSV is frequently observed in small vessels and EDV reduction is greater than PSV [54]. The CRA, as a smaller caliber vessel, is more sensitive to fluctuations in perfusion pressure, mostly due to IOP change [55]. The reduction of EDV may be a result of increased vascular resistance, following either IOP elevation or an increase of the vessel wall tension. In our study, there was no difference in IOP values between the groups, so lower EDV of the CRA in NTG patients might serve as an indirect marker of increased vascular tone following vasospasm.

We did not observe any significant changes in PSV, EDV, or RI of OA. The OA, unlike the CRA and SPCA, is a large-caliber vessel, relatively resistant to changes in IOP [56]. Parallel changes in both PSV and EDV of the OA have rarely been observed. In healthy subjects, flow velocities were reduced in response to hypoxia. Hypoxia did not induce PSV and EDV changes of OA in glaucoma patients, possibly because of preexisting vasospasm. Increase of EDV and decrease of RI in the OA were reported in NTG patients in response to hypercapnia, which also supports the hypothesis of preexisting vasospasm partially reversed under hypercapnia [53].

In NTG patients, amplitude of maximum hyperemic response (MAX) was negatively correlated with TSPCA RI. It has been demonstrated that repeated episodes of perfusion instability may contribute, via neurogenic and endothelial-mediated factors, to changes in vessel reactivity and loss of dilatory capacity. As observed in our study, the reduction of MAX amplitude with a parallel increase of TSPCA RI may suggest a possible link between systemic vascular dysregulation and impaired hemodynamics of some retrobulbar vessels in NTG. Whether the temporal quadrant of the retina is the predisposed region of endothelial dysfunction needs to be elucidated.

When interpreting our findings, some limitations of the study design should be considered. First, only 1 technique for assessing peripheral ED was applied. However it should be emphasized that the major advantage of LDF technique is its sensitivity in detecting and quantifying relative changes in skin blood flow in response to provocation. The results of LDF are also less observer-dependent as compared to the FMD method. Second, the measurement of baseline blood flow using CDI provides much less information about the vascular regulation characteristics of ONH blood vessels than the measurement of the hemodynamic response to the provocation. Third, some subjects were on antihypertensive therapy and, although both study groups did not differ in antihypertensive medications and the examined subjects did not take any medications at least 6 h before the examination, the impact of systemic drugs on autonomic function and hemodynamic outcomes should be considered. Most currently used antihypertensive drugs have been found to attenuate postsynaptic vascular sympathetic tone in hypertensive patients, whereas diuretics can increase sympathetic tone [57].

Conclusions

1. Patients with normal-tension glaucoma demonstrated prolonged post-occlusive hyperemia response compared to healthy subjects.
2. The results of the vascular response measured by LDF technique depended on the volume of measured tissue.
3. Amplitude of hyperemic response was negatively correlated with the resistance index of temporal short-posterior ciliary arteries.
4. Systemic endothelial dysfunction that leads, among other factors, to impaired control of perfusion within the microcirculation, may play an important role in the pathogenesis of NTG.

Acknowledgements

The authors would like to gratefully acknowledge Dr Janusz Sierdziński from Department of Informatics and Telemedicine, Medical University of Warsaw for his statistical analysis in this study.

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References:

1. Hayreh SS: The role of age and cardiovascular disease in glaucomatous optic neuropathy. Surv Ophthalmol, 1999; 43(Suppl.)1: S27–42
2. Douglas GR: Pathogenetic mechanisms of glaucoma not related to intraocular pressure. Curr Opin Ophthalmol, 1998; 9: 34–38
3. Flammer J, Orgul S: Optic nerve blood-flow abnormalities in glaucoma. Prog Retin Eye Res, 1998; 17: 276–89
4. Kerr J, Nelson P, O'Brien C: A comparison of ocular blood flow in untreated primary open angle glaucoma and ocular hypertension. Am J Ophthalmol, 1998; 128: 42–51
5. Yamamoto T, Kitazawa Y: Vascular pathogenesis of normal-tension glaucoma: a possible pathogenetic factor, other than intraocular pressure, of glaucomatous optic neuropathy. Prog Retin Eye Res, 1998; 17: 127–43
6. Hayreh SS, Podhajsky P, Zimmermann MB: Role of nocturnal arterial hypotension in optic nerve head ischemic disorders. Ophthalmologica, 1999; 213: 76–96
7. Hayreh SS: Factors influencing blood flow in the optic nerve head. J Glaucoma, 1997; 6: 412–25
8. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR: Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. Invest Ophthalmol Vis Sci, 2008; 49: 5412–18
9. Flammer J, Konieczka K, Flammer AJ: The primary vascular dysregulation syndrome: implications for eye diseases. EPMA J, 2013; 4: 14
10. Chowdhary S, Vaile JC, Fletcher J: Nitric oxide and cardiac autonomic control in humans. Hypertension, 2000; 36: 264–69
11. Kahler J, Mendel S, Weckmuller J et al: Oxidative stress increases synthesis of big endothelin-1 by activation of the endothelin-1 promoter. J Mol Cell Cardiol, 2000; 32: 1429–37
12. Martins Ferreira H, Nedergaard M, Nicholson C: Perspectives on spreading depression. Brain Res Brain Res Rev, 2000; 32: 215–34
13. Taniguchi T, Shimazawa M, Sasaoka M et al: Endothelin-1 impairs retrograde axonal transport and leads to axonal injury in rat optic nerve. Curr Neurovasc Res, 2006; 3: 81–88
14. He S, Prasanna G, Yorio T: Endothelin-1-mediated signaling in the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in astrocytes. Invest Ophthalmol Vis Sci, 2007; 48: 3737–45
15. Lip PL, Felmeden DC, Blann AD et al: Plasma vascular endothelial growth factor, soluble VEGF receptor FLT-1, and von Willebrand factor in glaucoma. Br J Ophthalmol, 2002; 86: 1299–302
16. Fadini GP, Pagano C, Baesso I et al: Reduced endothelial progenitor cells and brachial artery flow-mediated dilation as evidence of endothelial dysfunction in ocular hypertension and primary open-angle glaucoma. Acta Ophthalmol, 2010; 88: 135–41
17. Grieshaber MC, Flammer J: Blood flow in glaucoma. Curr Opin Ophthalmol, 2005; 16: 79–83
18. Flammer J, Mozaffarieh M: What is the present pathogenetic concept of glaucomatous optic neuropathy? Surv Ophthalmol, 2007; 52(Suppl.2): S162–73
19. Haefliger I O, Flammer J, Luscher TF: Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery. Invest Ophthalmol Vis Sci, 1992; 33: 2340–43
20. Gherghel D, Orgul S, Dubler B et al: Is vascular regulation in the central retinal artety altered in persons with vasospasm? Arch Ophthalmol, 1999; 117: 1359–62
21. Liebert A, Leahy M, Maniewski R: Multichannel laser-Doppler probe for blood perfusion measurements with depth discrimination. Med Biol Eng Comput, 1998; 36: 740–47
22. Resch H, Garhofer G, Fuchsjäger-Mayrl G et al: Endothelial dysfunction in glaucoma. Acta Ophthalmol, 2009; 87: 4–12
23. Buckley C, Hadoke PWF, Henry E, O'Brien C: Systemic vascular endothelial cell dysfunction in normal pressure glaucoma. Br J Ophthalmol, 2002; 86: 227–32
24. Henry E, Newby DE, Webb DJ, O'Brien C: Peripheral endothelial dysfunction in normal pressure glaucoma. Invest Ophthalmol Vis Sci, 1999; 40: 1710–14
25. Su WW, Cheng ST, Hsu TS, Ho W: Abnormal flow-mediated vasodilation in normal-tension glaucoma using a noninvasive determination for peripheral endothelial dysfunction. Invest Ophthalmol Vis Sci, 2006; 47: 3390–94
26. Holowatz LA, Thompson-Torgerson CS, Kenney WL: The human cutaneous circulation as a model of generalized microvascular function. J Appl Physiol, 2008; 105: 370–72
27. Gush RJ, King TA, Jayson MI: Aspects of laser light scattering from skin tissue with application to laser Doppler blood flow measurement. Phys Med Biol, 1984; 29: 1463–76
28. Łukasiewicz P, Liebert A, Zbieć A, Maniewski R: Evaluation of integrating probes in laser-Doppler perfusion measurements. Biocyb Biomed Engin, 2000; 4: 77–88
29. Dakak N, Husain S, Mulcahy D et al: Contribution of nitric oxide to reactive hyperemia: impact of endothelial dysfunction. Hypertension, 1998; 32: 9–15
30. Moens AL, Goovaerts I, Claeys MJ, Vrints CJ: Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? Chest, 2005; 127: 2254–63
31. Félétou M: Calcium-activated potassium channels and endothelial dysfunction: therapeutic options? Br J Pharmacol, 2009; 156: 545–62
32. Lorenzo S, Minson CT: Human cutaneous reactive hyperaemia: role of BKCa channels and sensory nerves. J Physiol, 2007; 585: 295–303
33. Polak K, Luksch A, Berisha F et al: Altered nitric oxide system in patients with open-angle glaucoma. Arch Ophthalmol, 2007; 125: 494–98
34. Galassi F, Renieri G, Sodi A et al: Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma. Br J Ophthalmol, 2004; 88: 757–60
35. Zhao DY, Cioffi GA: Anterior optic nerve microvascular changes in human glaucomatous optic neuropathy. Eye, 2000; 14: 445–49
36. Henry E, Newby DE, Webb DJ et al: Altered endothelin-1 vasoreactivity in patients with untreated normal-pressure glaucoma. Invest Ophthalmol Vis Sci, 2006; 47: 2528–32
37. Wang L, Fortune B, Cull G et al: Endothelin B receptor in human glaucoma and experimentally induced optic nerve damage. Arch Ophthalmol, 2006; 124: 717–24
38. Cleary C, Buckley CH, Henry E et al: Enhanced endothelium derived hyperpolarising factor activity in resistance arteries from normal pressure glaucoma patients: implications for vascular function in the eye. Br J Ophthalmol, 2005; 89: 223–28
39. Karnafel W, Juskowa J, Maniewski R et al: Microcirculation in the diabetic foot as measured by a multichannel laser Doppler instrument. Med Sci Monit, 2002; 8(7): MT137–44
40. Jarm T, Kragelj R, Liebert A et al: Postocclusive reactive hyperemia in healthy volunteers and patients with peripheral vascular disease measured by three noninvasive methods. Adv Exp Med Biol, 2003; 530: 661–69
41. Hirata K, Nagasaka T, Noda Y: Partitional measurement of capillary and arteriovenous anastomotic blood flow in the human finger by laser-Doppler-flowmeter. Eur J Appl Physiol Occup Physiol, 1988; 57: 616–21
42. Morales F, Graaff R, Smit AJ et al: How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: application of a standardized protocol to patients with peripheral arterial obstructive disease. Microvasc Res, 2005; 69: 17–23
43. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR: Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol Sci, 2006; 27: 503–8
44. Stewart J, Kohen A, Brouder D et al: Noninvasive interrogation of microvasculature for signs of endothelial dysfunction in patients with chronic renalfailure. Am J Physiol Heart Circ Physiol, 2004; 287: H2687–96
45. Boignard A, Salvat-Melis M, Carpentier PH et al: Local hyperemia to heating is impaired in secondary Raynaud's phenomenon. Arthritis Res Ther, 2005; 7: R1103–12
46. Binggeli C, Spieker LE, Corti R et al: Statins enhance postischemic hyperemia in the skin circulation of hypercholesterolemic patients: a monitoring test of endothelial dysfunction for clinical practice? J Am Coll Cardiol, 2003; 42: 71–77
47. Ruano J, Lopez-Miranda J, Fuentes F et al: Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol, 2005; 46: 1864–68
48. Wong BJ, Wilkins BW, Holowatz LA, Minson CT: Nitric oxide synthase inhibition does not alter the reactive hyperemic response in the cutaneous circulation. J Appl Physiol, 2003; 95: 504–10

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49. Higashi Y, Sasaki S, Nakagawa K et al: Effect of the angiotensin-converting enzyme inhibitor imidapril on reactive hyperemia in patients with essential hypertension: relationship between treatment periods and resistance artery endothelial function. J Am Coll Cardiol, 2001; 37: 863–70
50. Kragelj R, Jarm T, Erjavec T at al: Postocclusive reactive hyperemia test in patients with peripheral vascular disease and in healthy volunteers monitored by near infrared spectroscopy and laser Doppler flowmetry. Med Biol Eng Comp, 1999; 37(Suppl.1): 214–15
51. Colantuoni A, Bertuglia S, Intaglietta M: Biological zero of laser Doppler fluxmetry: microcirculatory correlates in the hamster cheek pouch during flow and no flow conditions. Int J Microcirc Clin Exp, 1993; 13: 125–36
52. Tagawa T, Imaizumi T, Endo T et al: Role of nitric oxide in reactive hyperemia in human forearm vessels. Circulation, 1994; 90: 2285–90
53. Hosking SL, Harris A, Chung HS et al: Ocular haemodynamic responses to induced hypercapnia and hyperoxia in glaucoma. Br J Ophthalmol, 2004; 88: 406–11
54. Kaiser HJ, Schoetzau A, Stümpfig D, Flammer J: Blood-flow velocities of the extraocular vessels in patients with high-tension and normal-tension primary open-angle glaucoma. Am J Ophthalmol, 1997; 123: 320–27
55. Galassi F, Sodi A, Ucci F: Ocular hemodynamics and glaucoma prognosis: a color Doppler imaging study. Arch Ophthalmol, 2003; 21: 1711–15
56. Harris A, Joos K, Kay M: Acute IOP elevation with scleral suction: effects on retrobulbar haemodynamics. Br J Ophthalmol, 1996; 80: 1055–59
57. de Champlain J: Do most antihypertensive agents have a sympatholytic action? Curr Hypertens Rep, 2001; 3: 305–13

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