Systemic Vascular Dysregulation and Retrobulbar Hemodynamics in Normal-Tension Glaucoma

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**Purpose.** To investigate the influence of systemic vascular dysregulation on retrobulbar hemodynamics in normal-tension glaucoma (NTG).

**Methods.** Forty-four untreated patients with NTG and 40 healthy controls matched for age, sex, and intraocular pressure were included. Cold-induced nailfold capillaroscopic features, mean and diastolic ocular perfusion pressures (mOPP, dOPP), endothelin-1 (ET-1) and nitric oxide markers, as nitrates (NO2), plasma values were recorded. Peak-systolic velocity, end-diastolic velocity (EDV), and resistivity index (RI) were measured in the ophthalmic artery (OA), short posterior ciliary arteries, and the central retinal artery by color Doppler imaging. Differences between groups were determined by Student’s *t*-test. Relationships among ET-1, NO2, OPP, and retrobulbar hemodynamics were assessed using correlation and multiple linear regression analyses.

**Results.** Altered capillaroscopy was more frequent (72.7% vs. 5.0%, *P* < 0.001), mOPP and dOPP values were lower (44.54 ± 2.81 vs. 52.18 ± 4.47 mm Hg; 57.89 ± 4.50 vs. 68.28 ± 6.91 mm Hg; *P* < 0.001), and ET-1 and NO2 values were respectively higher and lower (1.62 ± 0.22 vs. 1.12 ± 0.20 pg/mL and 142.17 ± 14.34 vs. 235.50 ± 6.16 μmol/mg prot; *P* < 0.001) in patients than the same values in controls. EDV was lower and RI higher in OA (5.87 ± 1.17 vs. 11.41 ± 2.50 cm/s; 0.76 ± 0.03 vs. 0.64 ± 0.03; *P* < 0.001) in subjects with NTG than in controls. In patients RI-OA was positively related to ET-1 (*t* = 2.704, *P* = 0.010) and negatively related to NO2 (*t* = −4.477, *P* < 0.001).

**Conclusions.** Impaired retrobulbar hemodynamics may proceed from a vascular endotheliopathy in patients with NTG.

The vascular theory postulated that vascular risk factors are pathogenetically relevant in glaucoma. A reduced ocular blood flow (OBF) is a major contributor to the onset and progression of glaucomatous optic neuropathy (GON), mainly in normal-tension glaucoma (NTG). In particular, studies by color Doppler imaging (CDI) demonstrated impaired retrobulbar hemodynamics in NTG.

The exact nature of the perturbation of OBF is still unclear, but a disturbed vascular autoregulation seems to be mostly involved. Vascular dysregulation is the inability of a tissue to maintain a constant blood supply despite changes in perfusion pressure. Primary vascular dysregulation (PVD) syndrome, the major cause of altered vascular autoregulation, is related to an imbalance of endothelium-derived vasoregulatory factors (EDVs). The main EDVs are nitric oxide (NO) and endothelin-1 (ET-1), which are released in all vessels, but exert their vasoactive properties, respectively dilation and contraction, mainly on small vessels, as retrobulbar ones. PVD is also often associated with low blood pressure (BP).

Systematic arterial hypotension, low ocular perfusion pressure (OPP), PVD, and impaired OBF may play a role in the pathogenesis of GON in NTG. The interrelationships among these factors have not yet been clearly established.

In the present study we evaluated the above-mentioned factors, to investigate their influence on retrobulbar hemodynamics in patients with NTG.

**Methods.**

The study was conducted on newly diagnosed, untreated, Caucasian NTG patients, referred to our glaucoma service between 2005 and 2008, and on healthy controls, matched for age, sex, and ethnicity, recruited from among spouses and friends of patients. One eye of each subject was considered for statistical analysis. In the patient group, selected eyes had a similar cup-to-disc ratio (CDR) and visual field indexes. In controls, the study eye was randomly chosen. The procedures conformed with the Declaration of Helsinki and the local ethical committee of the University of Florence (Florence, Italy). All the participants gave their written consent to the study procedures and to the use of their anonymous data for scientific purposes.

The following exclusion criteria were applied: cardiovascular and any systemic diseases that could interfere with the production and metabolism of NO; smoking habit; therapy with nonsteroidal anti-inflammatory drugs, NO donors, steroids, or angiotensin-converting enzyme inhibitors; myopia or hyperopia > 3 diopters and astigmatism > 1.5 diopters; ocular diseases other than glaucoma; central corneal thickness (CCT) < 550 μm or > 550 μm.

All the study procedures were conducted in each subject on the morning of the same day. Patients and controls were previously submitted to a diurnal intraocular pressure (IOP) curve, which consisted of seven IOP measurements at 2-hour intervals (8:00 AM to 8:00 PM) and showed IOP values < 22 mm Hg at all time points. They also underwent a 24-hour blood pressure monitoring that did not demonstrate any significant variation during the day and the night or nocturnal fluctuations > 20% or < 10%.

Diagnosis of PVD was based on clinical features and specific indicators (cold-induced altered capillaroscopy, elevated ET-1 plasma levels).

A questionnaire focused on the main symptoms and signs of PVD (migraine, cold extremities, and Prinzmetal’s angina) was given. An
electrocardiographic evaluation and a CDI examination were performed for the detection of myocardial ischemia, even silent, and carotid artery disease.

An ophthalmologic examination, including best-corrected Snellen visual acuity (BCVA), Goldmann tonometry, anterior and posterior segment slit-lamp examination, gonioscopy, CCT measurement, and visual field testing with a 24-2 full-threshold program (Humphrey Instruments, San Leandro, CA) were then performed by the two investigators (BG and FG). Functional defects were classified as stage 1L according to the Glaucoma Staging System 2 in the patient group; the latter expressed by nitrates (NO2) and cyclic guanosine monophosphate (cGMP).

Nailfold capillaroscopy was performed in all subjects in a room with a temperature between 24 and 25°C, after a 20-minute acclimatizing period. We used a microscope connected to a color television monitor and a video recorder (MS-500B, micro-SCOPEMAN handheld video microscope imaging system; Moritex Corp., Tokyo, Japan). We recorded three consecutive observations: at baseline, after immersion of the hand in a water bath of 40°C, and after cooling of nailfold surface at 14°C by rapidly decompressing carbon dioxide. We assumed an altered capillaroscopy response when nailfold blood flow was absent for at least 12 seconds after cold stimulation had been interrupted.37

Results are shown as mean ± SD. Statistical analysis was performed using computer analytical software (SPSS, version 18; SPSS Inc., Chicago, IL). Student’s t-test for unpaired data and χ2 test were used for comparisons between groups. Results were confirmed by means of other nonparametric tests for medians and distributions. The relationships among variables were analyzed by correlation and linear regression analyses. To avoid multicollinearity issues, we standardized the variables involved in the regression model. A value of P ≤ 0.05 was regarded as significant.

**Results**

Forty-four patients with NTG (24 males, 20 females; mean age, 64.45 ± 6.91 years) and 40 controls (22 males, 18 females; mean age, 62.75 ± 7.37 years) were enrolled. There were no significant differences between patients and controls in BCVA, CCT, and IOP. Mean CDR was higher in the NTG group than in controls (P = 0.001). Plasma levels of ET-1 and NO2 were higher in NTG than in controls (P = 0.001). Demographic and clinical characteristics of the study population are listed in Table 1.

As shown in Table 2, cold extremities, migraine, and altered nailfold capillaroscopy after a cold provocation test were highly prevalent in patients than in controls (P < 0.001), whereas Prinzmetal angina, myocardial ischemia, and carotid artery disease were absent in the whole population under study.

The two study groups differed in arterial blood pressure and ocular perfusion pressure values, which were significantly lower in patients (P < 0.001). Plasma levels of ET-1 and NO markers were respectively higher and lower in patients compared with controls (P < 0.001). Summary statistics are reported in Table 2.

ET-1 plasma values proved not to be correlated either with NO2 (r = −0.065, P = 0.675 for patients and r = −0.161, P = 0.321 for controls) or with cGMP (r = −0.062, P = 0.690 for patients and r = −0.212, P = 0.189 for controls).

Table 3 details the results of CDI investigation in the two study groups. In NTG eyes mean PSV-OA, EDV-OA, PSV-SCAs, EDV-SCAs, and EDV-CRA were lower in NTG eyes (P < 0.001)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 44)</th>
<th>Controls (n = 40)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold extremities, n (%)</td>
<td>16 (36.4)</td>
<td>2 (5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Migraine, n (%)</td>
<td>20 (45.5)</td>
<td>4 (10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Altered capillaroscopy, n (%)</td>
<td>32 (72.7)</td>
<td>2 (5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129.10 ± 7.01</td>
<td>139.20 ± 9.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75.91 ± 4.53</td>
<td>1971.13 ± 7.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mOPP, mm Hg</td>
<td>44.54 ± 2.81</td>
<td>1971.13 ± 4.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sOPP, mm Hg</td>
<td>111.50 ± 7.44</td>
<td>1971.13 ± 6.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dOPP, mm Hg</td>
<td>57.89 ± 4.30</td>
<td>1971.13 ± 6.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ET-1 plasma levels, pg/mL</td>
<td>1.62 ± 0.22</td>
<td>1971.13 ± 0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO2 plasma levels, μmol/ml/prot</td>
<td>142.17 ± 14.34</td>
<td>1971.13 ± 6.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cGMP plasma levels, pmol/ml/prot</td>
<td>1195.19 ± 88.17</td>
<td>1971.13 ± 134.90</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
for all comparisons), whereas mean RI-OA, RI-SPCAs, and RICRA were higher (P < 0.001 for both comparisons) than those recorded in control eyes.

Results in Tables 1, 2, and 3 were confirmed by nonparametric tests, not shown because they did not provide additional information.

RI-OA was higher in patients with altered nailfold capillaroscopy than that in subjects with normal examination (0.76 ± 0.03 vs. 0.74 ± 0.01, P = 0.011). Conversely, no significant differences in RI-OA values were found between subjects with or without cold extremities and migraine (0.77 ± 0.04 vs. 0.76 ± 0.03, P = 0.394 and 0.75 ± 0.03 vs. 0.77 ± 0.03, P = 0.154).

The correlations between RI-OA and the endothelial mediator (BP and OPP) values are detailed in Table 4.

Among the PVD markers, we included in the multiple linear regression analysis ET-1 because its higher values were found in patients with altered nailfold capillaroscopy (1.66 ± 0.19 pg/mL vs. 1.49 ± 0.23 pg/mL, P = 0.015). Given the very high correlation between dOPP and DBP (r = 0.936, P < 0.001) and between NO2 and cGMP (r = 0.867, P < 0.001), to avoid multicollinearity issues, we selected dOPP and NO2 values, instead of both dOPP and DBP and both NO2 and cGMP, respectively (Table 5).

Multiple regression analysis showed that ET-1 and NO2 maintained their significant association with RI-OA, even when controlling for the effect of the other independent variables. On the contrary, the effect of dOPP on RI-OA did not attain clinical significance.

Given the known association between ET-1 and NO and migraine, we performed all the analyses separately in patients with NTG, with and without migraine. Because we did not find any significant differences between the two groups in relation to the studied parameters, data are not shown.

Table 4. Correlation Analysis in Patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>r</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td>0.395</td>
<td>0.007</td>
</tr>
<tr>
<td>NO2</td>
<td>-0.573</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cGMP</td>
<td>-0.688</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP</td>
<td>0.002</td>
<td>0.988</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.342</td>
<td>0.022</td>
</tr>
<tr>
<td>mOPP</td>
<td>-0.243</td>
<td>0.112</td>
</tr>
<tr>
<td>sOPP</td>
<td>0.048</td>
<td>0.756</td>
</tr>
<tr>
<td>dOPP</td>
<td>-0.323</td>
<td>0.032</td>
</tr>
</tbody>
</table>

r, correlation coefficient.

Discussion

In the present study, we showed that systemic vascular dysregulation and impaired retrobulbar hemodynamics were more frequent in patients with NTG than in control subjects.

A large number of studies reported PVD, abnormal levels of EDVs, changes in OPP, and altered retrobulbar hemodynamics in NTG, 4-13,17-32,38-49 A likely basis for PVD is an endothelial dysfunction, which determines an imbalance in EDVs, with high ET-1 and low NO plasma levels. 17,27,35 Because they are the main determinants of optic nerve head (ONH) circulation, such an imbalance could negatively affect OBF, leading to constriction of retrobulbar arteries. 14-16

A peripheral endothelial dysfunction has been demonstrated in NTG. Henry et al. 45,46 described an altered vascular reactivity to EDVs by means of venous occlusion plethysmography, whereas Buckley et al.47 analyzed cutaneous artery biopsies. Su and colleagues48,49 provided evidence of a generalized endothelial dysfunction in patients with NTG, using the brachial artery ultrasound assessment of endothelium-dependent flow-mediated vasodilation. Given the anatomic and functional similarities between acral and ocular circulation, such a dysfunction could explain OBF abnormalities in NTG. 50-52 Vasospastic syndrome can negatively affect the OBF regulation, which cannot compensate for varying OPPs, leading to unstable ONH perfusion. 3,17-23,25,27 A disturbed blood flow regulation is the major link between PVD and GON, but how a condition of ocular vascular dysregulation can occur has yet to be fully elucidated.

Previous research focused separately on the influence of PVD, EDVs, and OPP on retrobulbar circulation in several types of glaucoma. Emre et al. 53 found an association between PVD and impaired retrobulbar hemodynamics in primary open- angle glaucoma (POAG); in contrast with ours, their data did not show differences in diagnosis of NTG and ET-1 values.

Our present results are in line with studies reporting decreased blood flow velocities and increased RI-OA in NTG patients with low NO or high ET-1 plasma and aqueous levels. 58,59 Anyway, the role of EDVs in the pathogenesis of NTG has not yet been directly demonstrated. 25

We confirmed the previously reported inverse correlation between OPP and OA circulation in POAG, NTG, and pseudoexfoliative glaucoma. 54-56 choroidal and CRA blood flow, fully directed to the eye, are influenced by IOP rather than by OPP changes. 37-39 Conversely, being only partially directed to the eye, but supplying the optic nerve, OA seems to be the most suitable vessel to investigate some vascular aspects of GON. 59

Literature data suggest that PVD could render the optic nerve more sensitive to stressful conditions, as low OPP. It has been shown that arterial hypotension increases the cutaneous vascular responsiveness to ET-1 in POAG and NTG subjects. 60 Similarly, low OPP might cause a significant reduction in ONH blood supply, leading to GON.

In the present study we tested the hypothesis that low NO, high ET-1, and low dOPP might cooperate in increasing resistivity in the ophthalmic artery of patients with NTG.

Table 5. Multiple Linear Regression Analysis in Patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>t</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td>2.704</td>
<td>0.010</td>
</tr>
<tr>
<td>NO2</td>
<td>-4.477</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dOPP</td>
<td>-1.001</td>
<td>0.323</td>
</tr>
</tbody>
</table>

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terms, we supposed that PVD would make the retrobulbar circulation more sensitive to low dOPPP.

Our multiple regression analysis brought out a definite influence of the imbalance of the NO/ET-1 system on RIOA. Although dOPPP did not attain statistical significance, a trend toward a negative interaction with EDVFs in determining RIOA was evident. Therefore, an endothelial dysfunction can provoke a reduction in OBF via an inappropriate retrobulbar vasoconstriction, suggesting an ONH vascular dysregulation in vasoconstrictive NTGs.

This study has limitations. It does not give a reason for the imbalance between ET-1 and NO plasma levels. Decreased NO production and altered receptor sensitivity can be alternative hypotheses. Of note, we did not find a correlation between NO and ET-1 values, but a possible explanation is beyond the aim of the present study. In addition, our investigation suggests that a vascular endotheliopathy can be the link between systemic vascular dysregulation and impaired retrobulbar hemodynamics in NTG, but does not provide evidence of a local endothelial dysfunction. In addition, no statistical power was given to justify our sample size because no related indications are currently available in the literature.

Further studies evaluating the direct effects of EDVFs in the ophthalmic circulation might help to clarify the pathogenesis of NTG and to develop targeted therapeutic interventions.

References


