Retinal Oximetry in Primary Open-Angle Glaucoma

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PURPOSE. To determine whether retinal vessel oxygen saturation is affected in primary open-angle glaucoma (POAG) patients.

METHODS. Retinal oxygen saturation in patients with POAG was measured in retinal vessels with a spectrophotometric retinal oximeter in darkness, and visual fields were obtained. Oxygen tension (PO₂) was calculated from oxygen saturation values. Statistical analysis was performed using Pearson’s correlation and Student’s t test.

RESULTS. Mean oxygen saturation in venules was higher in persons with poor visual fields (68% ± 4%, mean ± SD) than in those with good visual fields (62% ± 3%; P = 0.0018). The mean arteriovenous difference in oxygen saturation was lower in persons with poor visual fields (30% ± 4%, n = 9) than in those with good visual fields (37% ± 4%; P = 0.0003; n = 12). No correlation was found between saturation in retinal arterioles and visual field mean defect (n = 31; r = −0.16; P = 0.38). Oxygen saturation in retinal venules correlated positively with worsening visual field mean defect (r = 0.43; P = 0.015). Arteriovenous difference in oxygen saturation decreased significantly as the visual field mean defect worsened (r = −0.55; P = 0.0013). Mean PO₂ in venules was 38 ± 3 mm Hg. It was significantly higher in persons with poor visual field fields (40 ± 3 mm Hg) than in those with good visual fields (36 ± 2 mm Hg; P = 0.0016).

CONCLUSIONS. Deeper glaucomatous visual field defects are associated with increased oxygen saturation in venules and decreased arteriovenous difference in retinal oxygen saturation. The data suggest that oxygen metabolism is affected in the glaucomatous retina, possibly related to tissue atrophy. (Invest Ophthalmol Vis Sci. 2011;52:6409–6413) DOI:10.1167/iovs.10-6985

Glaucoma is considered to be an optic neuropathy associated with retinal ganglion cell death and visual field loss.1 Two long-proposed theories behind the pathogenesis of the disease involve separate mechanisms. One suggests that the physical consequences of increased intraocular pressure (IOP) directly result in tissue degeneration.2 The second theory proposes that vascular changes occur first and that an insufficient or poorly regulated blood supply leads to ischemia, hypoxia, and eventually tissue damage.3,4

There is growing evidence that blood flow in glaucomatous eyes is reduced or that its regulation is impaired compared with normal, nonglaucomatous eyes.5 For instance, blood flow deficiencies have been reported in the choroid6,7 and retrobulbar7–11 circulations in glaucoma patients.12–14 Plange et al.14 have shown that blood velocity in the central retinal artery is reduced in the more affected eye in asymmetric glaucoma. Sato et al.15 previously found that neuroretinal rim blood flow is reduced in areas corresponding to scotoma within asymmetric normal tension glaucomatous eyes. Additionally, systemic and localized vascular abnormalities have been linked to primary open-angle glaucoma (POAG).16–19 and reduced ocular perfusion pressure has been associated with both the prevalence20–22 and the incidence of glaucoma.23 All this evidence strongly indicates that vascular factors may play a role in glaucoma. Flammer et al.5,24 theorized that vascular autoregulation is impaired in glaucoma and results in impaired blood flow and energy metabolism.

It is unknown whether diminished blood flow causes or results from glaucomatous atrophy of ganglion cells and the optic nerve. In this study we used noninvasive spectrophotometric retinal oximetry25–27 to measure oxygen saturation in retinal arterioles and venules and to examine the correlation between these data and visual field defects in patients with POAG.

METHODS

The study was approved by the National Bioethics Committee of Iceland and The Icelandic Data Protection Authority and adhered to the tenets of the Declaration of Helsinki. All participants signed informed consent. The study was a prospective, nonrandomized clinical trial.

Eligible patients were recruited from our glaucoma clinic (Augnærkælin Reykjavíkur, Reykjavík, Iceland). The inclusion criteria included a diagnosis of POAG, age 40 years or older, and a history of increased IOP. Patients receiving antihypertensive medication for elevated systemic blood pressure and those with cataracts were not excluded because of the prevalence of these conditions in the age group studied. However, patients with other ocular diseases were excluded as were patients with other systemic diseases, such as diabetes. In all, 31 patients were enrolled into the study.

The right eye in each patient was studied except in the case of low image quality from the oximeter, in which case oxygen saturation in the left eye was measured. Clinical data of the study group are shown in Table 1.

Noninvasive spectrophotometric oximetry was performed on the same day as the ophthalmologic evaluations. The oximeter (Fig. 1) (Oxymap ehf., Reykjavik, Iceland) consists of a fundus camera (CR6–45NM; Canon Inc., Tokyo, Japan) with an attached beam splitter (Multispec Patho-Imager; Optical Insights, Tucson, AZ) and a digital camera (SBIG ST-7E; Santa Barbara Instrument Group, Santa Barbara, California).

CA). The instrument (described in detail elsewhere) delivers two images at different wavelengths: 605 nm, which is sensitive to oxygen saturation, and 586 nm, which is not sensitive to oxygen saturation. A software algorithm automatically calculates the optical density (OD) of retinal vessels from the two acquired images according to the equation $OD = \log (I_b/I)$, where $I_b$ is light reflected by the background to the side of the vessel and $I$ is the light reflected from the vessel. The ratio of the OD at 605 nm and the OD at 586 nm is approximately inversely related to hemoglobin oxygen saturation. Measurements were made in first- and second-degree retinal arterioles and venules inferior and superior to the optic nerve head. All measurements were performed in the dark, but with infrared aiming light. Each subject spent 2 minutes in darkness before oximetry.

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Pupils were dilated with 1% tropicamide (Mydriacyl; S.A. Alcon-Couvreur N.V., Puurs, Belgium). When needed, this was supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL) and 0.5% proparacaine hydrochloride (Alcaine; S.A., Alcon-Couvreur N.V.).

All visual field testing was performed using a perimeter (Octopus 123; Interzeg AG, Schlieren, Switzerland) using program G1. For 22 patients, the visual field tests were performed on the same day as oximetry. For nine patients, perimetry was performed on the same day as oximetry, but no more than 5 months elapsed before oximetry was performed. We required the reliability factor of the visual field to be under 15%; one patient was excluded because of a frequency of false-positive answers, resulting in a reliability factor >15%. Because false-negative answers represent the status of the eye rather than the status of the patient, they did not count as an exclusion factor. Visual fields with a mean defect ranging from −2 dB to 2 dB were defined as good visual fields, and visual fields with a mean defect equal to or exceeding 10 dB were defined as poor visual fields.

IOP was measured using Goldmann applanation tonometry mounted on a slit lamp (Haag-Streit BQ 900; Haag-Streit International, Köniz, Switzerland) on the day oximetry was performed. Systolic and diastolic blood pressure (SP and DP, respectively) were measured using an automatic sphygmomanometer (HEM-705CP; Omron, Kyoto, Japan). Mean arterial pressure (MAP) was calculated as $MAP = \frac{1}{3} SP + \frac{2}{3} DP$. Mean ocular perfusion pressure was calculated as $\frac{2}{3} MAP - IOP$. Finger oximetry was performed using a pulse oximeter (Biox 5700; Ohmeda, Boulder, CO) with the probe placed on the index finger of the right hand.

$P_{O2}$ was calculated from saturation values with an online calculator (http://www.ventworld.com/resources/oxydisso/oxydisso.html) that uses a previously published method. Calculations were based on the hemoglobin dissociation curve, and standard conditions were assumed for $T = 37^\circ C$ and $P_{CO2} = 40$ mm Hg. The pH level for arterial blood was assumed to be 7.4. The pH level for venous blood was assumed to be 7.3. At high oxygen saturation values, the dissociation curve flattens, and calculated $P_{O2}$ values higher than 90 mm Hg were recorded as $>90$ mm Hg.

Statistical analysis was performed (PRISM, version 5.01; GraphPad Software Inc., La Jolla, CA). Pearson’s correlation was used to detect correlations between mean defect and oxygen saturation levels. Unpaired Student’s $t$-test was used to detect a difference between two groups and their saturation levels. For both analyses, $P \leq 0.05$ was considered statistically significant.

## Results

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18 women, 13 men</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>66 ± 14</td>
</tr>
<tr>
<td>Intraocular pressure, mean ± SD, mm Hg</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD, mm Hg</td>
<td>134 ± 24*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean ± SD, mm Hg</td>
<td>81 ± 15*</td>
</tr>
<tr>
<td>Perfusion pressure, mean ± SD, mm Hg</td>
<td>49 ± 12*</td>
</tr>
<tr>
<td>Finger oximetry, mean ± SD, %</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>No. using drugs for glaucoma</td>
<td>12</td>
</tr>
<tr>
<td>No. using drugs for high blood pressure</td>
<td>18</td>
</tr>
<tr>
<td>Latanoprost</td>
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</tr>
<tr>
<td>Latanoprost, dorzolamide + timolol</td>
<td>5</td>
</tr>
<tr>
<td>Timolol</td>
<td>1</td>
</tr>
<tr>
<td>Timolol + latanoprost</td>
<td>1</td>
</tr>
<tr>
<td>Latanoprost, dorzolamide</td>
<td>1</td>
</tr>
<tr>
<td>Brinzolamide, brimonidine</td>
<td>1</td>
</tr>
<tr>
<td>Brinzolamide, latanoprost + timolol</td>
<td>1</td>
</tr>
<tr>
<td>Latanoprost, brimonidine, timolol</td>
<td>1</td>
</tr>
<tr>
<td>Latanoprost, brinzolamide, timolol</td>
<td>1</td>
</tr>
<tr>
<td>Trabeculectomy</td>
<td>6</td>
</tr>
<tr>
<td>Shunt (Ahmed tube)</td>
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</tr>
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</table>

* Blood pressure measurements from two patients were unavailable.

Mean retinal oxygen saturation for all patients ($n = 31$) was $99\% \pm 4\%$ (mean ± SD) in arterioles. In venules, the mean oxygen saturation was $64\% \pm 5\%$, and the mean arteriovenous difference was $35\% \pm 5\%$ (Table 2).

We compared retinal oxygen saturation between patients with good visual fields ($n = 12$; defined as mean defect ranging from $−2$ dB to $2$ dB) and those with poor visual fields ($n = 9$; defined as mean defect $\geq 10$ dB). No statistical difference was found between oxygen saturation in arterioles for the two groups ($P = 0.6$; Fig. 2A; Table 2). Patients with poor visual fields had higher venular oxygen saturation values ($68\% \pm 4\%$) than those with good visual fields ($62\% \pm 3\%; P = 0.0018$; Fig. 2B; Table 2). Patients with poor visual fields had lower arteriovenous differences in saturation ($30\% \pm 4\%$) than patients with good visual fields ($37\% \pm 4\%; P = 0.0003$; Fig. 2B; Table 2). Patients with poor visual fields had significantly higher $P_{O2}$ in venules ($40 \pm 3$ mm Hg) than patients with good visual fields ($36 \pm 2$ mm Hg; $P = 0.0016$; Table 2).

In retinal arterioles, there was no statistical correlation with visual field mean defect ($r = -0.16; P = 0.38$; Fig. 3). The slope of the regression line for arterioles was $-0.082/\text{dB}$, with $95\%$ confidence intervals (CIs) ranging from $-0.27$ to $0.11 \text{ dB/}%$ (Fig. 3). Statistically significant correlation was
found between oxygen saturation in venules and visual field mean defects ($r = 0.43; P = 0.015$; Fig. 3) with the slope of the regression line at 0.28%/dB, where 95% CIs ranged from 0.058 to 0.50%/dB (Fig. 3). A negative correlation was seen between arteriovenous difference in oxygen saturation and visual field mean defect ($r = -0.55; P = 0.0013; n = 51$; Fig. 3). The slope of the regression line for arteriovenous difference was $-0.36%/\text{dB}$, with 95% CIs from $-0.57$ to $-0.15%/\text{dB}$ (Fig. 3).

No statistical correlation was found between retinal oxygen saturation values and intraocular pressure, finger oximetry values, or perfusion pressure.

**DISCUSSION**

Oxygen saturation in the arterioles was stable and did not change with increasing visual field defect. However, oxygen saturation in venules increased as the visual field became worse (Figs. 2A, 3). Venous $\text{PO}_2$ was significantly higher in eyes with increased visual field defect (Table 2). The arteriovenous difference in retinal oxygen saturation decreased with increased visual field defect (Figs. 2B, 3). Lower arteriovenous difference, along with decreased ocular blood flow in glaucoma, as seen in earlier studies, suggests that less oxygen uptake by the retina is associated with an increased visual field defect. We cannot tell from the data exactly what caused this. However, in atrophic tissue, oxygen consumption would be decreased, which could reduce the proportion of oxygen extracted from the blood.

There seemed to be no hypoxia in the retinal blood vessels in our glaucoma patients during measurements. If blood flow and oxygen delivery are insufficient for the oxygen demand of the tissue, the hypoxic tissue should extract an increased proportion of the oxygen content from the blood, and the oxygen saturation levels in venules should decrease, as should the $\text{PO}_2$ in tissue and vessels. This is not what we observed in this study. However, our data do not exclude ischemia and hypoxia from the pathogenesis of glaucoma. Ischemia and hypoxia may be intermittent. These conditions are more likely to be present when the glaucoma treatment is poor, when IOP spikes, or when blood pressure drops, as is often the case at night. Because our patients were under active glaucoma treatment and good IOP control at the time of the study (mean IOP, 17 ± 4 mm Hg), retinal hypoxia might have been prevented. Oximetry measurements in patients with progressive glaucoma whose IOP is poorly controlled and who have a greater likelihood of low perfusion pressure are needed to elicit additional information on the role of ischemia and hypoxia in the pathophysiology of glaucoma. We also do not know what happens in the glaucomatous eye before any visible or measured damage has happened to the optic nerve.

Two papers from other research groups have previously been published on noninvasive retinal oxygen saturation measurement in patients with glaucoma. Michelson and Scibor measured oxygen saturation in healthy persons and in patients with high or normal tension glaucoma. They found that arteriovenous differences in saturation decreased with smaller rim area of the optic nerve head. This finding is in agreement with our results, although the progression of glaucoma is measured by mean defect in the visual field in our study. Their data, though not statistically significant, also showed a trend for increased values of oxygen saturation in venules in high tension.
sion glaucoma. As in our study, they did not find a correlation between arteriolar oxygen saturation and mean defect or rim size in high-tension glaucoma. Ito et al.\textsuperscript{39} reported lower oxygen saturation level in retinal tissue in open-angle glaucoma patients compared with healthy controls. They also found a correlation between the visual field defect and oxygen saturation in the inferotemporal region of the retina in open-angle glaucoma eyes with high IOP, which is in agreement with our results. On the other hand, they did not measure a difference in oxygen saturation levels in the retinal artery and vein between healthy persons and those with glaucoma. They state that two-dimensional resolution is not precise enough to measure oxygen saturation of a defined point, such as an area over a retinal artery and a vein because of problems with eye movements and because the size of each pixel in their system was large. In addition, tissue oxygen saturation measurements are difficult because the influence of the choroid must be considered.\textsuperscript{39}

Oximetry data give an objective measurement that corresponds to the psychophysical data provided by perimetry. With further development and testing, this measurement may support visual field testing in glaucoma by providing objective data on POAG pathology and progression.

Our study represents an initial clinical study and is not without limitations. Some patients were taking glaucoma medications that could have affected oxygen saturation values (Table 1). For example, Trustason et al.\textsuperscript{40} measured lower arteriolar and venular oxygen saturation when patients switched from dorzolamide-timolol combination to timolol alone. Some patients had undergone glaucoma surgery (Table 1). Previous results from Hardarson et al.\textsuperscript{26} found a 2% increase in oxygen saturation in arterioles after surgery but no difference in venules or arteriovenous difference. However, we do not think this significantly influenced our results. An ideal study would have included optical coherence tomography with the oximetry and visual field measurements and would have included a group of age-matched, healthy controls. Direct measurements of retinal blood flow in addition to oximetry also would have improved the study. Finally, assessing the diurnal fluctuations in all the aforementioned parameters may provide a more complete understanding of retinal function and metabolism.

Light conditions during measurements must be considered. All subjects spent 2 minutes in darkness before oximetry, and it was dark during oximetry except for the flash of light when each photograph was taken. Stefansson et al.\textsuperscript{41,42} found that preretinal oxygen tension levels in monkeys and rabbits adapt quickly to changes in illumination. Linsenmeier\textsuperscript{43} measured oxygen responses in the dark-adapted cat retina under varying illumination conditions and found that changes of oxygen consumption are rapid and that retinal oxygen tension changes dramatically with illumination because of a light-induced decrease in photoreceptor oxygen consumption. Hardarson et al.\textsuperscript{27} measured oxygen saturation in humans with the same oximeter that was used in this study and found that changes in oxygen saturation were similar after 5 minutes or 30 minutes of dark adaptation. In that study, the time duration for dark adaptation did not seem to affect oxygen saturation readings for retinal vessels. Because our subjects underwent the same illumination conditions before measurements, all would have been affected the same way and would have responded quickly to changes in illumination according to the manner described. We did not, however, know completely how the flash could affect our results. The same amount of light was flashed onto the retina of each patient so the affect from it did not alter between them; therefore, the difference in oxygen saturation probably did not result from this.

Mean oxygen saturation values obtained in this study (Table 2) compared well with previous measurements by our group.\textsuperscript{25–27} and calculated $PO_2$ values were slightly higher but in the same range as measurements in experimental animals.\textsuperscript{44–46}

In summary, we found a correlation between worsening visual field defect and increasing venous oxygen saturation and decreasing arteriovenous difference. Patients with poor visual fields showed higher oxygen saturation and $PO_2$ values for venules and decreased arteriovenous difference than did those with good visual fields. This is consistent with tissue atrophy in glaucoma. Further clinical studies are needed to confirm and further expand the use of oximetry in glaucoma to enhance our understanding of its pathophysiology and, potentially, the clinical evaluation of glaucoma pathology and progression.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue), and arteriovenous difference (black) in patients with POAG. The slope of the regression line for arteriovenous difference was $-0.56\%$/dB, $0.26\%$/dB for venules, and $-0.082\%$/dB for arterioles. $n = 51$, $r$, correlation coefficient.}
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
Method & Oxygen Saturation & \textsuperscript{a} $PO_2$ (mm Hg) \\
\hline
\textit{In situ} & 87 & 80 - 85 \\
\hline
\textit{Ex situ} & 87 & 80 - 85 \\
\hline
\textit{In vivo} & 87 & 80 - 85 \\
\hline
\end{tabular}
\caption{Oxygen saturation comparison between \textit{in situ}, \textit{ex situ}, and \textit{in vivo} measurements.}
\end{table}

\textbf{References}


