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Retinal and choroidal oxygen saturation of the optic nerve head in open-angle glaucoma subjects by multispectral imaging

Gai-yun Li, PhD, Samer abdo Al-wesabi, PhD, Hong Zhang, MD*

Abstract

The aim of this study was to determine whether differences exist in oxygen supply to the optic nerve head (ONH) from the retinal and choroidal vascular layers in patients with primary open angle glaucoma (POAG) using multispectral imaging (MSI).This ia an observational, cross-sectional study.

Multispectral images were acquired from 38 eyes of 19 patients with POAG, and 42 healthy eyes from 21 matched volunteers with Annidis' RHA multispectral digital ophthalmoscopy. Superficial and deeper oxygen saturation of the optic disc was represented by the mean gray scale values on the retinal and choroidal oxy-deoxy maps, respectively. Statistical analysis was performed to detect differences in ONH oxygen saturation between the 2 groups. Oxygen saturation levels in the eyes of POAG patients with severe glaucoma were compared to those of fellow eyes from the same subjects. Linear correlation analysis was performed to assess the association between ONH oxygen saturation and systemic and ocular parameters.

No statistical difference was found in retinal and choroidal oxygen saturation between the POAG and control groups. In the glaucoma patients, retinal oxygen saturation was lower for eyes with worse visual fields than in those with good visual fields ($t=4.009$, $P=0.001$). In POAG patients, retinal oxygen saturation was dependent on mean defect of visual field and retinal nerve fiber layer thickness (RNFLT) ($r=0.511$, 0.504, $P=0.001$, 0.001, respectively), whereas the choroid vasculature oxygen saturation was inversely related to RNFLT ($r=-0.391$, $P=0.015$). An age-dependent increase in retinal oxygen saturation was found for both the POAG and control groups ($r=0.473$, 0.410, $P=0.007$, 0.003, respectively).

MSI revealed a significant correlation between functional and structural impairments in glaucoma and retinal oxygen saturation. MSI could provide objective assessments of perfusion impairments of the glaucomatous ONH. This is a preliminary indication of the effectiveness of MSI for studying POAG.

Abbreviations: CCT = central corneal thickness, EDI-OCT = enhanced depth imaging optical coherence tomography, IOP = intraocular pressure, MD = mean defect of visual field, MSI = multispectral imaging, ONH = optic nerve head, POAG = primary open angle glaucoma, RNFLT = retinal nerve fiber layer thickness, SE = spherical equivalent, VA = visual acuity.

Keywords: choroidal oxygen saturation, hypoxia, multispectral imaging, optic nerve head, primary open angle glaucoma, retinal oxygen saturation

1. Introduction

Glaucoma is the worldwide leading cause of irreversible vision loss.[1] In addition to elevated intraocular pressure (IOP), a growing body of evidence suggests that an abnormality of the optic nerve head (ONH) perfusion also plays an important role in primary open angle glaucoma (POAG) pathophysiology.[2–5]

The surface layer of the ONH receives blood from branches of the retinal arterioles. The rest of the nerve in front of the lamina cribrosa is supplied by branches from the peripapillary choroidal vessels. Ischemia and other abnormalities in either retinal or choroidal blood flow are potentially associated with impaired oxygen supply to the ONH. With technical developments, a dual-wavelength technique has been used for evaluating the retinal arteries and veins of subjects with POAG, and it has been revealed that a change in oxygen saturation occurs in glaucomatous retina vessels around the ONH.[6–11] However, at present, changes have only been observed in the large retinal vessels surrounding the optic disc, and this technique does not assess the oxygen supply to the optic nerve directly. Moreover, very little is known about the relationship between POAG and choroidal oxygen saturation. Thus, it is crucial that we evaluate the role of the superficial and deeper circulatory disorders of the ONH in glaucoma.

To address this question, we used multispectral imaging (MSI) technology to measure oxygen saturation in the retinal and choroidal vessels simultaneously in POAG patients and normal controls, to determine whether ONH oxygen saturation in patients with glaucoma is associated with retinal nerve fiber layer changes and visual field defects.

Editor: Iok-Hou Pang.

The authors report no conflicts of interest.

This work was supported by the National Natural Science Foundation of China. Grant 81471744. None of the authors acknowledge any conflict of interest in connection with this submission.

This manuscript was edited by a professional medical editor at Editage.

Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China.

* Correspondence: Dr. Hong Zhang, Department of Ophthalmology, Tongji Hospital, 1095 Liberation Road, Wuhan 430030, China (e-mail: dr_zhanghong@vip.163.com).

Medicine (2016) 95:52(e5775)

Received: 8 April 2016 / Received in final form: 4 December 2016 / Accepted: 6 December 2016

http://dx.doi.org/10.1097/MD.0000000000005775

2. Methods

2.1. Subjects

The study was performed according to the tenets of the Declaration of Helsinki. This study was approved by the ethics committee of Tongji Hospital and informed consent was signed by all subjects. All subjects were examined at Tongji Hospital, Wuhan, Hubei, China, from May 2015 until August 2015. Thirty-eight eyes of 19 patients with POAG, and 42 healthy eyes of 21 matched volunteers were consecutively enrolled in the study. All subjects received a complete ophthalmic examination, which included determination of the best-corrected visual acuity (VA), IOP (Goldmann tonometer; Haag-Streit, Koeniz, Switzerland), gonioscopy, ocular fundus examination, visual field test (Humphrey, 30–2, SITA – standard; Carl Zeiss Meditec, Dublin, California, USA), measurement of central corneal thickness (CCT; Visante OCT; Carl Zeiss Meditec, Dublin, CA), dioptometry, determination of the thickness of the peripapillary retinal nerve fibre layer (RNFLT; SD-OCT; Heidelberg Engineering GmbH, Heidelberg, Germany), and MSI (RHA, Annidis, Ottawa, Canada) examination. The VA was measured using the ETDRS regimen and was converted to the logarithm of the minimum angle of resolution (LogMAR) units for statistical analysis.

The inclusion criteria were: open iridocorneal angle; glaucomatous optic disc changes; visual field defects consistent with glaucoma. The exclusion criteria were: advanced cataract; a history of intraocular surgery; serious systemic diseases such as vascular disease, hypertension, or diabetes mellitus.

Based on Hoddap-Parrish-Anderson criteria, the stages of glaucoma were classified according to the visual field mean defect (MD). The stages were: mild (MD ≥ -6 dB), moderate (-12 dB $\leq$ MD < -6 dB), or severe (MD < -12 dB). Glaucoma patients continued the use of all antiglaucomatous medications throughout the study. Beta-blockers (n = 25), prostaglandin derivatives (n = 23), and alpha-2-selective adrenergic agonists (n = 9) were used as monotherapy (n = 17) or in combination (2 drugs: n = 17, 3 drugs: n = 2). Two eyes did not receive any IOP-lowering treatment.

2.2. MSI measurement procedures

Multispectral images were obtained using an Annidis' RHA multispectral digital ophthalmoscope. Images of the right and then the left eye were taken in a darkroom. The flash intensity setting was set at 50 Ws. The imaging mode used was the Full Spectrum mode. Eleven monochromatic fundus images, centered at the optic disc, were obtained at 11 wavelengths ranging from 550 to 850 nm. The retinal and choroidal oxy-deoxy maps were automatically generated by combining images taken at 2 wavelengths (580 and 590 nm, and 760 and 810 nm, respectively). The distribution of oxygen saturation in the retinal and choroidal blood was determined from the oxy-deoxy maps, and used in further analysis.

2.3. Measurements of oxygen saturation

Oxygen saturation values were calculated using the Image J software (version 1.42, National Institutes of Health). Briefly, we visually determined the ONH margin, and an ellipse was drawn manually on the retinal and choroidal oxy-deoxy map images. The superficial and deeper oxygen saturation levels of the optic disc were represented by the mean gray scale values of the ONH on the retinal and choroidal oxy-deoxy maps, respectively. White represents a high saturation value (Fig. 1). Measurements were conducted twice by 2 separate observers who remained masked to the patients' status.

2.4. Reliability of the MSI technology

In 10 eyes of 10 healthy subjects, the retinal and choroidal oxygen saturation of ONH was measured 5 times within 1 day by 1 observer. One eye of each subject was randomly selected for evaluation. The intraclass correlation coefficient or reliability

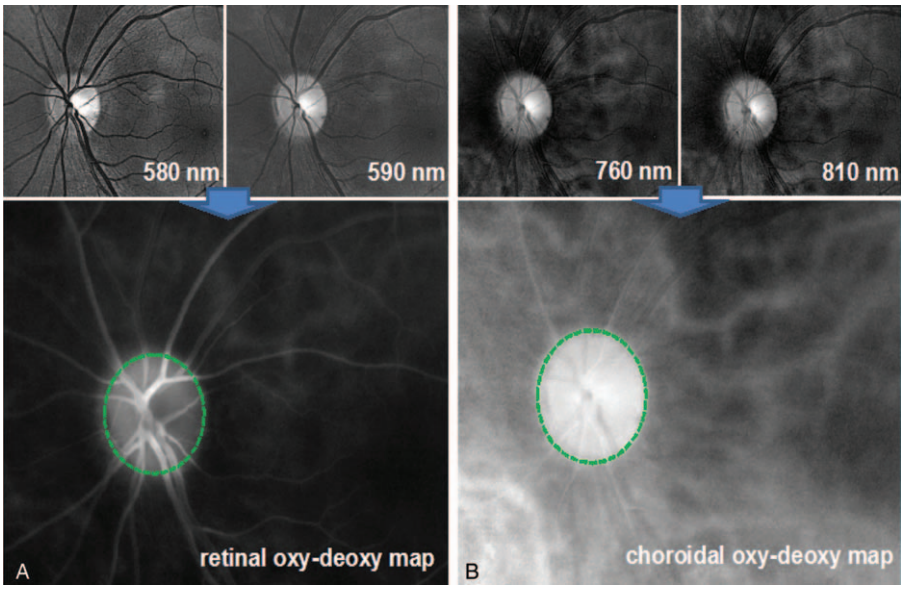

Figure 1. Oxy-deoxy maps of a healthy volunteer obtained using an Annidis' RHA multispectral digital ophthalmoscope. The retinal oxy-deoxy map (A) was obtained by combining 580 nm and 590 nm wavelength images. The choroidal oxy-deoxy map (B) was obtained from 760 nm and 810 nm images. The measurements were performed within an ellipse bounding the optic nerve head margin.

Table 1

Reliability of repeated measurements of ONH oxygen saturation.

One Eye/Subject	Retinal Saturation (Pixels) (n=5)		Chroidal Saturation (Pixels) (n=5)	
	Mean	SD	Mean	SD
1	119.8	6.9	200.8	12.8
2	156.8	1.6	191.4	23.4
3	169.6	7.5	196.0	4.3
4	144.8	3.3	182.2	17.1
5	149.6	2.6	162.6	14.0
6	153.2	5.9	176.2	24.3
7	125.0	4.1	214.4	2.9
8	141.8	3.9	194.6	1.1
9	199.8	2.8	129.0	5.4
10	185.2	5.5	148.4	3.4

ONH = optic nerve head, SD = standard deviation.

coefficient α of oxygen saturation was calculated using Cronbach method. Cronbach alpha was determined for 95% confidence interval (SPSS 12.0; SPSS Inc, Chicago, IL).

2.5. Statistical analysis

SPSS 12.0 was used for the statistical analyses. The oxygen saturation levels of the ONH at the retinal and choroidal layers were compared between patients with POAG and normal subjects using an independent sample *t* test. For POAG patients, the ONH oxygen saturation levels of eyes with severe glaucoma were compared to those of fellow eyes from the same patients, using a paired-samples *t* test. The same test was used to compare the oxygen saturation in the right and left eyes of normal subjects. Linear correlation analysis was performed to assess the possible association between ONH oxygen saturation and systemic and ocular parameters in glaucoma patients and control subjects, respectively. A P value of <0.05 was considered statistically significant.

3. Results

The intraclass reliability coefficient α of the instrument under the previously described conditions was 0.99 in retinal saturation and 0.95 in choroidal saturation. Table 1 lists mean and standard deviation data for repeated measurements on the same eye. Thus, measurements of retinal and choroidal oxygen saturation of ONH fell within a good range of reliability.

Of the 38 eyes with POAG, 18 had mild glaucoma, 6 had moderate glaucoma, and 14 had severe glaucoma. The subjects' clinical characteristics and results of the ONH oxygen saturation analysis are presented in Table 2. The glaucoma patients and healthy controls did not significantly differ in age, sex, spherical equivalent (SE), or IOP. Eyes with POAG had a significantly reduced VA and visual field, and lower RNFLT, compared to the control subjects. There was no statistically significant difference in both retinal oxygen saturation ($t=0.751$; $P=0.455$) and choroidal oxygen saturation ($t=0.700$; $P=0.486$) in the ONH between the 2 groups.

In the glaucoma patients, the ONH oxygen saturation in the retina was lower for eyes with severe visual field defects compared to those with mild visual field defects ($t=4.009$; $P=0.001$) (Fig. 2), and there was no significant difference in choroidal vasculature ($t=-1.029$; $P=0.317$). In the healthy control subjects, there was no significant difference between both eyes in either retinal or choroidal saturation ($t=1.135$, -0.042; $P=0.263$, 0.967, respectively).

Linear correlation analysis demonstrated that ONH oxygen saturation in the retinal vasculature was associated significantly with the MD, RNFLT, and age ($r=0.511$, 0.504, 0.473, $P=0.001$, 0.001, 0.003, respectively) (Fig. 3), whereas ONH oxygen saturation in the choroidal vasculature showed no association with the MD or age. Conversely, choroidal vasculature oxygen saturation was inversely related to the RNFLT ($r=-0.391$, $P=0.015$). These results are summarized in Table 3. In healthy volunteers, a statistically significant correlation was only observed between the retinal oxygen saturation of the ONH and subject age ($r=0.410$, $P=0.007$).

Table 2

Description of the study groups.

Parameter	Patients With POAG	Healthy Controls	*P*
Number	19	21	
Age, y	44.7±15.0	41.0±9.6	0.199
Sex (f/m)	8/11	8/13	0.796
VA (logMAR)	0.2±0.3	0.0±0.0	0.002
SE (D)	−1.59±2.98	−1.49±1.89	0.853
IOP, mmHg	16±4	14±3	0.184
CCT, μm	533±29	524±26	0.160
MD, dB	−12.18±10.74	−2.10±1.74	0.000
RNFLT, μm	65.76±22.12	106.69±9.71	0.000
Retinal saturation (pixels)	139.74±24.38	143.38±18.82	0.455
Choroidal saturation (pixels)	183.32±25.89	187.06±21.91	0.486
MAP, mmHg	92.6±11.2	89.3±5.7	0.110
OPP, mmHg	46.2±6.7	44.9±4.5	0.306

Data were presented as mean±standard deviation. CCT = central corneal thickness, f/m = female/male, IOP = intraocular pressure, MAP = mean arterial pressure, MD = visual field mean defect, OPP = ocular perfusion pressure, POAG = primary open-angle glaucoma, RNFLT = retinal nerve fiber layer thickness, SE = spherical equivalent, VA = visual acuity.

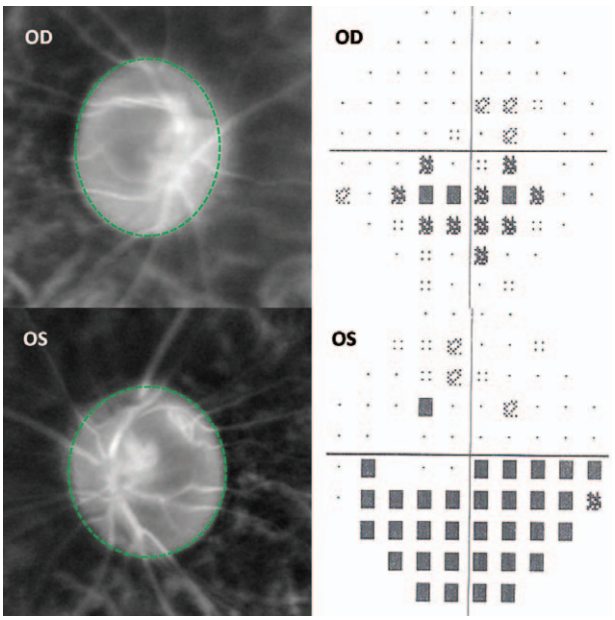

Figure 2. The optic nerve head retinal oxy-deoxy maps of a patient with primary open angle glaucoma. The gray scale measurement shows a lower retinal oxygen saturation (average gray scale value155.33 pixels) in the left eye (OS) with severe visual field defects, and a higher oxygen saturation (average gray scale value175.00 pixels) in the right eye (OD) with mild visual field defects.

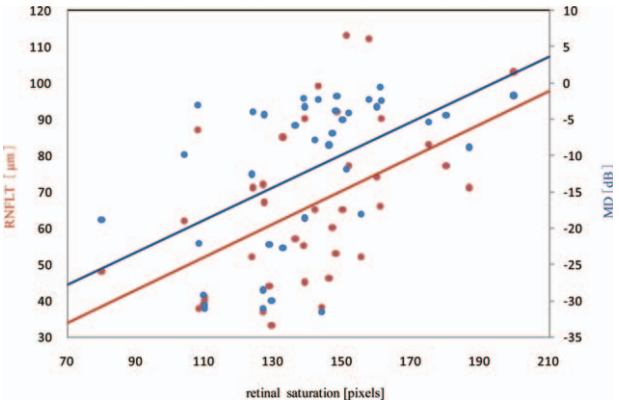

Figure 3. Dependence of retinal saturation on thickness of the retinal nerve fibre layer and visual field mean defect in patients with glaucoma. Pearson correlation coefficients: retinal saturation and RNFLT (red): $r=0.504$, $P=0.001$, retinal saturation and MD (blue): $r=0.511$, $P=0.001$. dB = decibel, MD = visual field mean defect, RNFLT = retinal nerve fibre layer thickness.

4. Discussion

Recently, there has been renewed interest in the use of dual-wavelength techniques for the study of associations between POAG and retinal oxygen saturation.[6–11] Owing to limitations in the available measurement tools, there have been few studies investigating the relationship between choroidal oxygen saturation and POAG. This study demonstrates a correlation between both ONH retinal and choroidal circulatory oxygen saturation and the severity of POAG.

MSI is an emerging technique that uses different light wavelengths, ranging from 550 to 850 nm, to examine the layers of the retina and choroid progressively. MSI is noninvasive and does not require mydriasis. MSI not only has the advantages of being quick, repeatable, and applicable in a primary care setting, but also has the ability to map retinal and choroidal vasculature oxygen levels by measuring oxygenation of the blood.[12] This approach has perhaps demonstrated a high discriminative value in the study of some eye diseases, such as vein occlusion, polypoidal choroidal vasculopathy, Stargardt disease, and hydroxycholoroquinetoxicity.[12–15]

Using dual-wavelength techniques, previous studies involving oxygen saturation measurements in glaucoma patients have demonstrated an increased retinal venous oxygen saturation and a decreased arterivenous oxygen difference in glaucomatous eyes with advanced visual field defects, compared to those with milder or no defects.[8,11,16] However, each of these studies only measured oxygen saturation in the large retinal vessels around the optic disc, and did not assess the optic nerve oxygen supply directly. The ONH in particular is mainly nourished by microcirculatory networks, rather than large vessels. Therefore, oxygen saturation values obtained directly from the ONH, which were used for the generation of oxy-deoxy maps in this experiment, could be more meaningful.

We found no significant differences in the ONH oxygen saturation measurements in retinal or choroidal oxy-deoxy maps between the POAG and control groups. However, in the glaucoma group, retinal oxygen saturation of the ONH in eyes with advanced glaucoma was significantly lower than that in fellow eyes from the same patients. In this study, the oxygen saturation measurement relies on analysis of reflected light. As a result, fundus pigmentation, cataract, and ocular perfusion pressure can cause artefactual changes in saturation measurements.[17,18] All of these factors differed between patients with POAG and the healthy controls. Therefore, in patients with unilateral advanced glaucoma, the severely affected eye was compared to the fellow eye of the same subject. This model is therefore advantageous, in that the effects of the above factors on our results are minimized. Comparing MSI oxy-deoxy maps from the same patient could easily be used to identify unilateral differences in oxygen supply.[13] The results presented here show that gray scale measurements of the ONH taken from a retinal oxy-deoxy map in eyes with a worse visual field were significantly lower than that of fellow eyes from the same patients. Thus, these findings indicate an association between a decreased oxygen supply to the ONH and glaucomatous optic neuropathy, at least at the surface nerve fiber layer.

This study also demonstrated that a decrease in the retinal oxygen saturation of the ONH correlated with a worsening of the visual field as well as thinning of the retinal nerve fiber layer in glaucoma patients. A possible explanation for this observation could be inadequate perfusion of the ONH, which is directly responsible for glaucomatous damage. This finding is consistent with results obtained by Jia et al.[2,3] Using the split-spectrum amplitude-decorrelation angiography algorithm, Jia et al found that ONH perfusion is significantly reduced in glaucomatous eyes, compared to normal eyes. If the ONH is subjected to reduced perfusion owing to POAG, subsequent hypoxia as a result of the decrease in blood flow could be expected. Similarly, under the pathological conditions applied in our study, retinal oxygen saturation of the ONH was found to be deficient. Our results, in conjunction with the studies mentioned above, provide indirect evidence that indicates blood flow and retinal oxygen levels are decreased in POAG, and low ONH vessel oxygen saturation most likely corresponds with decreased ONH blood

Table 3

Linear correlation analysis for variables associated with ONH oxygen saturation in POAG.

	Retinal Vasculature		Choroidal Vasculature	
Parameter	***r***	***P***	***r***	***P***
Age, y	0.473	0.003	−0.073	0.663
SE (D)	0.088	0.600	−0.150	0.870
IOP, mmHg	0.199	0.232	−0.044	0.791
CCT, μm	−0.118	0.479	0.066	0.693
MD, dB	0.511	0.001	−0.265	0.108
RNFLT, μm	0.504	0.001	−0.391	0.015

CCT = central corneal thickness, IOP = intraocular pressure, MD = visual field mean defect, POAG = primary open-angle glaucoma, RNFLT = retinal nerve fiber layer thickness, *r* = correlation coefficient, SE = spherical equivalent.

flow. Therefore, an increased severity of glaucoma might be accompanied by a reduction in oxygen supply to the ONH. This could indicate that decreased oxygen delivery and blood flow are a primary cause, rather than a consequence, of optic nerve degeneration.

The blood supply of the ONH can be divided into 4 regions, from the anterior to posterior aspects: surface nerve fiber layer, prelaminar region, lamina cribrosa region, and retrolaminar region.[19] As the prelaminar region is supplied by branches from the peripapillary choroidal vessels, ischemia and abnormalities in choroidal blood flow may play a role in the pathophysiology of glaucomatous optic neuropathy. Because of the unavailability of a safe and reliable noninvasive technology for oxygen studies in the choroid, there are few reports from published studies assessing the link between oxygen saturation and glaucoma that have investigated choroidal oxygen saturation. Kristjansdottir et al[20] reported that dual-wavelength (570 and 600 nm) oximetry is sensitive to changes in oxygen saturation in both choroidal and retinal vessels, but no previous studies have definitively established a relationship between choroidal blood flow and glaucoma. Histopathological studies have provided evidence that eyes with advanced glaucoma have reduced density in the choriocapillaris and large choroidal vessels.[21,22] The ONH may receive less oxygen from the choroid in POAG, according to studies that indicate decreased choroidal blood flow.[16,17] However, in this study, we found no differences between the choroidal oxygen saturation of the ONH in POAG patients compared to healthy controls, or between eyes with advanced glaucomatous damage and fellow eyes of the same patients. No significant correlation was found between choroidal oxygen saturation and MD. Our findings are in agreement with other studies measuring peripapillary choroidal thickness. Using enhanced depth imaging optical coherence tomography (EDI-OCT), other authors found no significant differences in choroidal thickness around the optic nerve between glaucoma patients and healthy controls.[23,24] However, our results indicate that ONH oxygen saturation in choroidal circulation decreases significantly with increasing RNFLT. We speculated that the choroidal oxygen saturation values obtained in this study are related to retinal transmission function rather than glaucomatous damage as determined by RNFLT. These findings might indicate that choroidal oxygen saturation of the ONH is not a sufficient measure for use in evaluating the damage from glaucomatous optic neuropathy, but the possibility of primary involvement of choroidal tissue hypoxia cannot be excluded completely.

There was no significant difference in age between the patients and controls. An age dependent increased retinal oxygen saturation of the ONH was found in both the POAG and control group, although the mechanisms behind this are unknown. Previous work has shown a significant age-related decline of RNFLT in both healthy individuals[25] and POAG patients.[26] The cause of this age-related thinning of the nerve fiber layer is attributed to the loss of retinal ganglion cells and a reduction in number of axons.[27] Therefore, we could expect to observe a decrease in oxygen extraction with aging. The reason for the observed higher oxygen saturation in older subjects could be a reduction in oxygen consumption, owing to the loss of neuronal tissue in both healthy individuals and those suffering from glaucoma, as occurs normally with aging.

The clinical application of MSI is still in its initial stages. There are several limitations to our study that should be mentioned. First, as discussed above, the absolute oxygen saturation values are unknown in this experiment; the oxygen saturation level is represented by a gray scale in the oxy-deoxy maps. Kramer et al[28] was able to demonstrate a linear relationship between the oxygen saturation of blood and the absorption of red and infrared wavelengths of light. Their technique of measuring the blood oxygen saturation using an isobestic and oxygen-sensitive wavelength is the basis of the MSI system. The MSI system can be calibrated using data acquired from normal subjects. Second, patients were taking different glaucoma medications. The observed effects of glaucoma drugs on the ocular blood perfusion are not consistent, and their effects on retinal oxygen saturation remain to be evaluated.[6,7,29,30] In particular, Siesky et al[7] suggested that brinzolamide and dorzolamide may increase retinal oxygen saturation in patients with glaucoma. In this study, no POAG patients were taking brinzolamide and dorzolamide to lower their IOP. Third, the sample size was relatively small. Future multicenters, larger-sample, randomized controlled trials might be able to show diagnostic power better than this study.

5. Conclusion

We established a correlation between the retinal oxygen saturation of the ONH, and visual field MD as well as RNFLT. Changes in the choroidal oxygen saturation of the ONH did not reveal signs of ischemic effects on choroidal circulation. MSI is capable of evaluating the oxygen levels of the ONH using retinal and choroidal oxy-deoxy maps and is therefore a promising tool that will provide additional information in our understanding of the intriguing interplay between the retinal and choroidal vasculature in both normal optic nerve function and glaucomatous optic neuropathy.

Acknowledgments

The authors thank Dr. He Lu for her help with statistical analysis.

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