• Clinical Research •

Hemoglobin oxygen saturation in myopic eyes: a scanning laser ophthalmoscope study

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Abstract

● **AIM:** To evaluate retinal hemoglobin oxygen saturation in myopic eyes by scanning laser ophthalmoscope (SLO) and to assess its correlations with different severity of myopia.

● **METHODS:** Sixty-one eyes from 61 patients were included and subdivided into three groups according to their refractive errors: high myopia group [20 eyes, spherical equivalent (SE)≤-6 D]; low and moderate myopia (22 eyes, -6.0<SE≤-0.5 D); normal (19 eyes, -0.5<SE<+0.5 D). All subjects underwent SLO imaging with dual lasers (532 nm and 633 nm). The oxygen saturations of hemoglobin in arteries (SO_2A) and veins (SO_2V), and their differences (SO_2AV) were estimated from the optical densities of the vessels on the images at the two wavelengths. Pearson's or Spearman's rank correlation coefficient were calculated to assess the correlation between retinal hemoglobin oxygen saturation and refractive error/axial length (AL).

● **RESULTS:** For the retinal oxygen saturation, the SO_2V in high myopia group (73.21%±21.42%) was significantly higher than that in normal group (55.81%±21.69%) and low and moderate myopia group (56.88%±13.83%, P<0.05). The SE was significantly correlated with SO_2A (r=-0.30) and SO_2V (r=-0.36; P<0.05), and AL was also significantly correlated with SO_2A (r=0.27) and SO_2V (r=0.30; P<0.05). No significant correlations were found between SO_2AV and SE and AL (P>0.05).

● **CONCLUSION:** SO_2A and SO_2V increased in more myopic eye based on SLO measurements. Further studies are warranted to investigate the changes of retinal hemoglobin oxygen saturation in myopia with different methods.

● **KEYWORDS:** myopia; retina; hemoglobin oxygen saturation; scanning laser ophthalmoscope

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INTRODUCTION

Myopia is one of the most common ocular disorders worldwide[1-2]. Previous study estimated that the global prevalence of myopia would increase to 39.9% in 2030 and 50% in 2050[3]. The development of myopia was accompanied by the increase of the axial length (AL), which can cause choroidal and retinal atrophy, resulting in high incidence of choroidal neovascularization, glaucoma, and macular retinoschisis[3-6]. Visual impairment caused by the secondary retinal lesions would influence the quality of life and increase financial burdens[7-8].

Previous studies reported the retinal hemodynamic changes in myopic eyes, including the decrease of retinal vascular diameter and blood flow volume[4,9-14]. The retinal hemodynamic changes are important in the development of myopia and myopic retinopathy. Yet, the causality between myopia and retinal hemodynamic changes remains unclear. Among all the biometrics of retinal hemodynamics, the oxygen saturation represents oxygen supply, which is one of the most important functions of retinal vessels. With the development of non-invasive retinal oximetry, it enabled the measurement of oxygen supply in the retina[15-19]. Non-invasive retinal oximetry measures the differences in the light absorption between oxyhemoglobin and deoxyhemoglobin[20-21]. It can noninvasively detect the oxygen saturation of retina and has been used to study the potential mechanism of ischemic retinopathy and atrophic retinopathy[15-19].

The correlations between the retinal oxygen saturation and the progression of myopia have been investigated. However, the relationship of the retinal hemoglobin oxygen saturation and myopia was controversial. Liu *et al*[22] demonstrated higher arteriolar oxygen saturation in higher myopic eyes in teenagers. Conversely, lower arteriolar oxygen saturation in adult myopic eyes was also observed[23-25], indicating that the correlations between arteriolar and venular oxygen saturation in myopic eyes still need to be confirmed. Most of these studies used two commercially available instruments for oximetry (Oxymap[26], Iceland or Imedos[27], Germany) with the dual-wavelength digital fundus camera. These devices are based on the traditional fundus cameras with limited view of retina, high requirements for pupils, and susceptible to refractive media opacity[28]. The pupil dilation by the mydriatic eye drops may affect retinal hemodynamics[25]. Instead, the scanning laser ophthalmoscope (SLO; Daytona Optos, Dunfermline, Scotland, the United Kingdom) can acquire non-mydriatic ultra-high-resolution images with a view of up to 200 degrees of the retina in less than 0.4s, by using two different wavelength lasers (532 nm and 633 nm)[29]. The peculiarity of using the dual-wavelength laser made it possible to measure retinal oxygen saturation. Previous studies have shown that the SLO can produce accurate and repeatable results for retinal oxygen saturation[28,30-31].

Due to the conflicting results on the changes of the retinal hemoglobin oxygen saturation in myopic eyes, it is worthwhile to use a different device to clarify the relationship between retinal hemoglobin oxygen saturation and myopia. To our knowledge, no study has used SLO to investigate retinal oxygen saturation in myopia. Herein this study aimed to evaluate the correlations of retinal oxygen saturation with different severity of myopia using the SLO system.

PARTICIPANTS AND METHODS

Ethical Approval This retrospective study has been approved by the Human Medical Ethics Committee of the Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong [EC20200609(6)-p23], which was in accordance with the tenets of the Declaration of Helsinki. In this retrospective study, written informed consent for participation was not required in accordance with the national legislation and the institutional requirements.

Participants Sixty-one eyes from 61 consecutive healthy subjects who underwent routine ophthalmic examination and met the inclusion criteria were included. All study subjects underwent the imaging by an SLO system during November 2015 to December 2021. All subjects also received complete ophthalmic examinations, including the uncorrected visual acuity and the best corrected visual acuity, intraocular pressure, AL (IOLMaster700, Carl Zeiss Meditec Germany), non-dilated fundus stereoscopic examination. All study subjects received visual acuity test, including the uncorrected visual acuity and the best corrected visual acuity, by an experienced optometrist with a phoropter. Subjective refraction was performed without cycloplegia. Spherical equivalent (SE) was calculated as (SE=sphere+0.5×cylinder) and used to categorize the refractive status. All study subjects were grouped according to SE. Normal, low and moderate myopia, and high myopia were defined as $-0.5<SE<+0.5$ D, $-6.0<SE\leq-0.5$ D, and $SE\leq-6.0$ D.

The exclusion criteria included: 1) age<18y; 2) best corrected visual acuity <0.8 (Snellen visual acuity); 3) myopic refractive error increased over 0.5 D in 2 recent years; 4) tilted optic disc or choroid/retina atrophy; 5) opacity of refractive media; 6) a history of retinopathy which may affect the retinal hemodynamics in addition to myopia; 7) a history of medications which may affect the hemodynamics of the eye; 8) a history of intraocular surgeries or refractive surgeries; 9) a history of smoking and high blood pressure.

Scanning Laser Ophthalmoscope Imaging The SLO imaging was carried out by the SLO Daytona. All examinations were performed by a single experienced operator. "Optomap Color" mode was selected in the Image Modalities. The Auto-Focus mode was used during the imaging. The images, including the optomap images (optomap532 and optomap633), were collected from the device-specific software of Daytona (Figure 1). All images were cropped to 5×5 papillary diameter (PD) with the optic disc centered within the field of view. A circum-papillary region of interest (ROI) was defined, extending between one and two optic disc radii from the optic disc edge (Figure 2). The retinal vessels in the ROI were labeled by two clinical ophthalmologists (Xiao ZX and Zhou H) using Labelme ver.4.5.7 (Massachusetts Institute of Technology, Cambridge, Massachusetts, USA). The intraclass correlation coefficient was used for the consistency test. If the labeled results were controversial, the images were re-assessed by a senior ophthalmologist.

Retinal Hemoglobin Oxygen Saturation Measurement Retinal hemoglobin oxygen saturation was calculated by the optical density (OD) and optical density ratio (ODR) from the four largest arterioles and the four largest venules of ROI. The OD was calculated as $\log (I_{outside}/I_{inside})$[20-21]. I_{inside} represented the minimum pixel intensity in the blood vessel, which minimized the influence on the bright central reflex of the vessel. The $I_{outside}$ represented the average pixel intensity outside vessel, extending one vascular diameter from the vessel edge (Figure 3). The ODR were calculated as OD633/OD532, whereas the OD633 and OD532 represented the OD of retinal

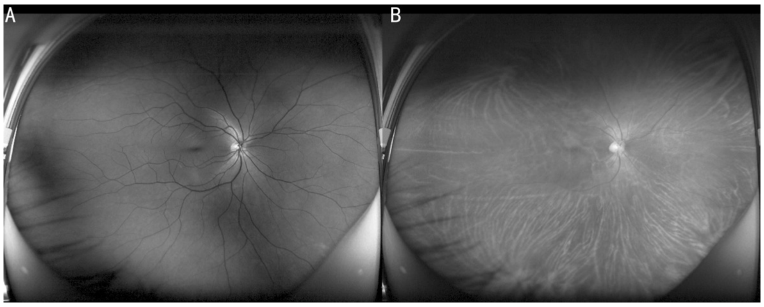

Figure 1 Optomap images of Optomap532 and Optomap633 A: The green Optomap image (532 nm); B: The red Optomap image (633 nm).

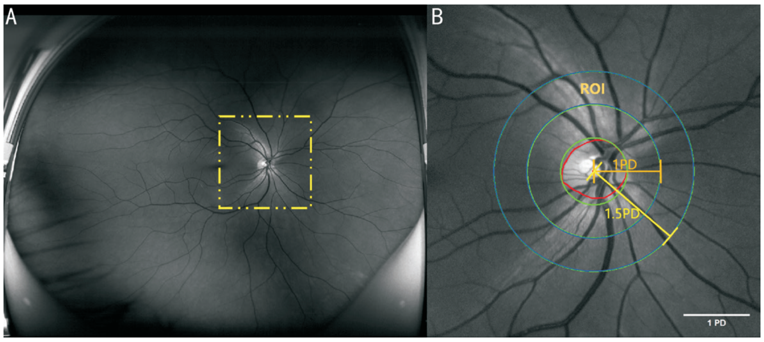

Figure 2 Optomap image preprocessing A: The original green Optomap image (532 nm); B: The red line labeled the optic disc, and the red dot represented the center of optic disc. The original images were cropped to 5×5 papillary diameter (PD) with the optic disc centered within the field of view. The circum-papillary region of interest (ROI) was defined, extending between one and two optic disc radii from the optic disc edge. The optic disc radii were defined as the maximum radius of optic disc. The ROI was shown by the blue line.

vessels in opto633nm and opto532nm images, respectively. Previous studies indicated that the ODR had a linear relationship with the retinal hemoglobin oxygen saturation[20-21] [$SatO_2$=(a×ODR+b)×100%, Formula 1]. According to the correction model of Schweitzer *et al*[21], the average oxygen saturation of retinal artery is 92.2%, and that of retinal vein is 57.9%. The average ODR of the normal group was obtained and substituted into Formula 1 to get constant a and b. The oxygen saturations of hemoglobin in arteries (SO_2A), in veins (SO_2V), and the difference between SO_2A and SO_2V (SO_2AV) were calculated by the Formula 1.

Statistical Analysis Statistical analysis was performed using commercially available software (SPSS ver. 26.0; SPSS Inc., Chicago, IL, USA). Data distribution for normality was checked using the Shapiro-Wilk's test. According to the data normality of the groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis H test was used to compare the age, AL, and retinal hemoglobin oxygen saturation among different groups. Multiple testing correction between groups was adjusted by the Tukey-Kramer test. χ^2 test was used for the comparisons on gender and laterality. Pearson's correlation coefficients or Spearman's rank correlation coefficient were calculated to assess the correlation between retinal hemoglobin oxygen saturation and refractive error and AL. $P<0.05$ was considered as statistically significant.

RESULTS

Demographics of Participants Sixty-one eyes from 61 patients were included in this study, including 19 eyes in the normal group, 22 eyes in the low and moderate myopia group, and 20 eyes in high myopia group. The demographics of the study subjects are shown in Table 1. The mean ages of the normal, low and moderate myopia, and high myopia groups were 29.89±5.54y, 25.32±5.90y, and 26.25±5.10y respectively. The mean age of the study subjects in the low and moderate myopia group was significantly younger than that in the other groups (*P*=0.01).

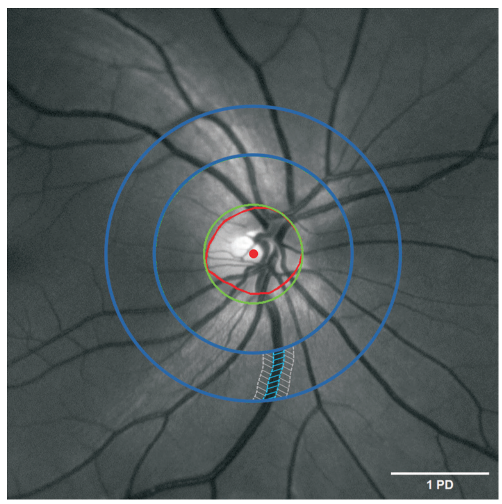

Figure 3 Retinal hemoglobin oxygen saturation measurement The blue dotted line marked the vein, and the blue diagonal area was the pixel intensity detection area inside the vessel. The gray diagonal area was the pixel intensity detection area outside the vessel.

Table 1 Demographics of study subjects mean±SD

Demographics	Normal (*n*=19)	Low and moderate myopia (*n*=22)	High myopia (*n*=20)	*P*
Age (y)	29.89±5.54	25.32±5.90	26.25±5.10	0.01[b]
Sex (male/female)	10/9	15/7	6/14	0.05[a]
Eye (right/left)	10/9	11/11	11/9	0.95[a]
SE (diopter)	0.01±0.03	-3.60±1.29	-7.91±1.64	<0.01[b]
AL (mm)	23.76±0.53	25.49±0.89	26.87±0.96	<0.01[b]

SD: Standard deviation; SE: Spherical equivalent; AL: Axial length. [a]Chi-Square test; [b]Kruskal-Wallis *H* test.

Table 2 Hemoglobin oxygen saturation in myopic eyes

Hemoglobin oxygen saturation	Emmetropia (*n*=19)	Low and moderate myopia (*n*=22)	High myopia (*n*=20)	*P*	P^a	P^b	P^c
SO_2A, %	88.70±21.41	92.78±19.81	102.38±19.36	0.10	0.79	0.09	0.28
SO_2V, %	55.81±21.69	56.88±13.83	73.21±21.42	0.01	0.98	0.02	0.02
SO_2AV, %	32.89±21.55	36.21±19.91	29.17±12.27	0.10	0.83	0.80	0.43

SO_2A: The oxygen saturations of hemoglobin in arteries; SO_2V: The oxygen saturations of hemoglobin in veins; SO_2AV: The difference between SO_2A and SO_2V. [a]Emmetropia *vs* low and moderate myopia; [b]Emmetropia *vs* high myopia; [c]Low and moderate myopia *vs* high myopia.

Mean AL of the normal, low and moderate myopia, and high myopia groups were 23.76±0.53 mm, 25.49±0.89 mm, and 26.87±0.96 mm respectively ($P<0.01$). Mean SE of these groups were 0.01±0.03 D, -3.60±1.29 D, and -7.91±1.64 D respectively ($P<0.01$). There were no statistically significant differences in gender and laterality among the three study groups.

Retinal Hemoglobin Oxygen Saturation in Myopic Eyes Mean SO_2A, mean SO_2V, and mean SO_2AV for all three studied group were 94.66%±20.63%, 61.90%±20.42%, and 32.87%±18.29%, respectively. The retinal hemoglobin oxygen saturation of the three studied groups are shown in Table 2. Mean SO_2A of the normal, low and moderate myopia, and high myopia groups were 88.70%±21.41%, 92.78%±19.81%, and 102.38%±19.36% respectively, whereas mean SO_2V of these groups were 55.81%±21.69%, 56.88%±13.83%, and 73.21%±21.42% respectively. Mean SO_2AV of the three studied groups were 32.89%±21.55%, 36.21%±19.91%, and 29.17%±12.27% respectively. There were significant differences among the three study groups in SO_2V ($P=0.01$). But no significant difference was found in SO_2A ($P=0.10$) and SO_2AV ($P=0.10$). The SO_2V was significantly higher in the high myopia group (73.21%±21.42%) as compared to the normal group (55.81%±21.69%, $P=0.02$) and low and moderate myopia group (56.88%±13.83%, $P=0.02$). No significant differences were found for the pairwise comparison of other groups in SO_2A and SO_2AV (all $P>0.05$). The analysis of covariance (ANCOVA) was employed, with age included as a covariate, to exclude the influence of age on the retinal hemoglobin oxygen saturation. The results of ANCOVA indicated that, after adjusting for age, the SO_2V values among different groups were still significantly different ($F=5.502$, $P=0.007$).

Table 3 The correlation of hemoglobin oxygen saturation with SE and AL *r* (*P*)

Variable	SO_2A^a	SO_2V^a	SO_2AV^b
SE	-0.30 (0.03)	-0.36 (<0.01)	0.02 (0.87)
AL	0.27 (0.04)	0.30 (0.02)	0.04 (0.78)

SO_2A: The oxygen saturations of hemoglobin in arteries; SO_2V: The oxygen saturations of hemoglobin in veins; SO_2AV: The difference between SO_2A and SO_2V; SE: Spherical equivalent; AL: Axial length. [a]Pearson correlation coefficients; [b]Spearman's rank correlation coefficient.

Significant correlations were found between SE and SO_2A ($r=-0.30$, $P=0.03$) as well as SO_2V ($r=-0.36$, $P<0.01$). Moreover, significant correlations were also found between AL and SO_2A ($r=0.27$, $P=0.04$) as well as SO_2V ($r=0.30$, $P=0.02$). No significant correlation was found between SE and SO_2AV ($r=0.02$, $P=0.87$), as well as between AL and SO_2AV ($r=0.04$, $P=0.78$; Table 3).

DISCUSSION

This study investigated the retinal hemoglobin oxygen saturation in myopic eyes by the SLO system. Results from this study revealed that the SO_2A and SO_2V in the myopic eyes increased with the severity of myopia and AL in the retinal hemoglobin oxygen saturation. The SO_2V was significantly higher in the high myopia group as compared to the normal group and low and moderate myopia group.

The current study found similar results to the prior studies based on the fundus camera[9,22,32-33]. Previous studies reported an inconsistent relationship between the retinal hemoglobin oxygen saturation and the severity of myopia. Zheng *et al*[23] and Ge *et al*[24] found that the arteriolar oxygen saturation decreases with the severity of myopia in adult myopic eyes. Lim *et al*[25] also demonstrated lower arteriolar oxygen saturation in higher myopic and longer eyes. Man *et al*[34] and Zheng *et al*[23] found that the SO_2AV decreases with the severity of myopia and AL. In contrast, Liu *et al*[22] demonstrated higher retinal hemoglobin oxygen saturation in higher myopic eyes. Yang *et al*[35] did not find the direct relationship between the retinal hemoglobin oxygen saturation and the SE. The study subjects of Liu *et al*'s studies[22,36] were adolescents, while the Man *et al*'s[34], Zheng *et al*'s[23], and Lim *et al*'s[25] studies focus on the middle-aged and elderly people. The discrepancy of these previous studies could be due to the age of study subjects. Previous studies indicated age as an important factor in retinal hemoglobin oxygen saturation, and atrophy in the retina and choroid increases with age[37]. Jani *et al*[38] demonstrated that the retinal arterial and venous hemoglobin oxygen saturation decrease by 1.02% and 1.59% respectively, for each 10 years of age. Mean age of study subjects in the current study was similar to previous studies by Liu *et al*[22,36]. Furthermore, the SE of all study subjects increased less than 0.50 D in recent 2y. The subjects with choroidal/retinal atrophy or peripheral retinal degeneration have been excluded from this study. The age-related retina atrophy may progress quickly in myopic eyes with time, leading to lower retinal hemoglobin oxygen saturation as detected by Lim *et al*[25] and Zheng *et al*[23]. In current study, the SO_2A and SO_2V increased in more myopic eyes, which is similar to the Liu *et al*'s[22,36] study. The discrepancy of the current study with the Lim *et al*[25] and Zheng *et al*[23] studies could be due to the age of study subjects. Previous study found that the choroidal atrophy occurred earlier than retinal atrophy in the early stage of myopia[36]. The development of choroidal atrophy may lead to change of microenvironment related to oxygen supply. We postulated that the retina could adapt to this change of microenvironment by increasing oxygen supply in young myopia, leading to the increase of retinal hemoglobin oxygen saturation in retinal vessels. In current study, the high myopia group had greater increments in SO_2A and SO_2V among three study groups, indicating that this regulation is more significant in high myopia. Meanwhile, the increased AL would cause the thinning of the retina, resulting in easier oxygen diffusion from choroid into retina. However, the thinner retina would also result in lower level of oxygen consumption. The changes of retinal hemoglobin oxygen saturation in myopic eyes should account for the balance of these factors.

The current study found no significant correlation between SO_2AV and SE/AL, which was similar to that in Lim *et al*[25]. On the contrary, previous studies[22,37] found that the SO_2AV increases with SE and AL, while other studies[23,34] found that the SO_2AV decreased in myopic eyes. It was proposed that the increased SO_2AV reflects a higher level of oxygen consumption[23]. Previous study[24] found that retinal oxygen consumption would also change with age. Since the sample size was relatively small in currents study, we could not conclude the relationship between SO_2AV and myopia.

Previous studies were based on the fundus camera-based systems[22-23,25,34-36]. In contrast, an SLO system was adopted to investigate the retinal hemoglobin oxygen saturation in myopic eyes in current study. The SLO system relies on the dual monochromatic lasers to scan the retina with less light exposure, acquiring the image data with a large field of view up to 200° through an un-dilated pupil. Acquiring the ultra-wide field retinal images without the need of pupil dilation, the SLO system can investigate the retinal hemoglobin oxygen saturation without the mydriatic eye-drops and timely detect the peripheral retinal degeneration, which could lead to reduced oxygen requirements. However, the effect of mydriatic eye-drops and peripheral retinal degeneration was not considered in prior studies[22-23,25,34-36]. The difference of measuring method might have led to the inconsistent results with prior studies[22-23,25,34-36]. Further studies with larger sample sizes are warranted to determine the relationship between the change of retinal hemoglobin oxygen saturation and the development of myopia.

There were several limitations in this study. First, only one topical hemodynamic parameter was analyzed in current study. Both systemic and topical hemodynamic parameters can contribute to the development of myopia[9,39-41]. Further studies with more comprehensive measurements and optimized methods can help to clarify the hemodynamic changes in myopic eyes. Second, the standard deviations in the current study were larger than those in previous reports, and the SO_2A values in high myopia group was above 100%. This discrepancy may be due to the difference in the devices used. The hemoglobin oxygen saturation is a relative value, which represents the deviation from the normal value. The measurements in the current study were similar to the previous studies based on the SLO system, which obtained SO_2A values above 100%[28,30-31]. However, further studies are warranted to calibrate our new method. Lastly, the sample size was small in current study. We included only the subjects with healthy retina and choroid, to avoid the influence of choroidal/retinal changes on the measurement of oxygen saturation[25-26]. This led to a relatively small sample size in our study. It is warranted to proceed with further studies based on larger sample size.

In summary, this study revealed that the retinal hemoglobin oxygen saturation in both retinal vein and artery increased with the severity of myopia in healthy young adult. Further studies with different methods are warranted to clarify the changes of retinal hemoglobin oxygen saturation in different myopic stage.

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