Reliability and Determinants of Retinal Vessel Oximetry Measurements in Healthy Eyes

WanFen Yip,[1,2] Rosalynn Siantar,[1] Shamira A. Perera,[1] Nia Milastuti,[1] Kee Ka Ho,[1] Bernard Tan,[1] Tien Yin Wong,[1–3] and Carol Y. Cheung[1–3]

[1]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore
[2]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore
[3]Duke-NUS Graduate Medical School, Singapore

Correspondence: Carol Y. Cheung, Singapore Eye Research Institute, The Academia, 20 College Road, Discovery Tower Level 6, Singapore 169856; carol.cheung.y.l@seri.com.sg.

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Purpose. To assess the reliability and determinants of retinal vessel oximetry measurements with the Oxymap T1 Retinal Oximeter in normal Asian eyes.

Methods. Subjects older than 40 years without a history of stroke and heart disease were recruited from a community-based clinic. Subjects underwent standardized systemic and ocular examinations. Normal eyes were defined as eyes without major eye diseases such as age-related macular degeneration, glaucoma, or retinopathy. Retinal vessel oximetry levels were measured by using the Oxymap T1 Retinal Oximeter. Intra- and intergrader reliability of retinal vessel oximetry measurements were assessed by using 50 images. Intravisit repeatability of retinal vessel oximetry measurements was assessed by using 20 paired images. Univariable linear regression was performed to examine the associations between retinal vessel oximetry measurements and systemic determinants.

Results. A total of 118 retinal oximetry images were included in the final analysis. Intra- (intraclass correlation coefficient [ICC] values: 0.89–0.99) and intergrader (ICC values: 0.77–0.94) reliability, and intravisit (ICC values: 0.85–0.96) repeatability were both high. In the linear regression analysis, older age was associated with reduced overall retinal venular oximetry levels (β: −2.61%; 95% confidence interval [CI]: −4.92 to −0.29) and reduced inferior–nasal retinal venular oximetry levels (β: −3.53%; 95% CI: −6.07 to −0.99).

Conclusions. The Oxymap Retinal Oximeter allows reliable and repeatable retinal vessel oximetry measurements. Age is the main factor that influences retinal venular oximetry levels and should be taken into account when retinal oximetry measurements are interpreted.

Keywords: retinal oxygen saturation, functional imaging, retinal oximetry

The retina is one of the most metabolically active tissues in the human body[1] and consumes oxygen more rapidly than other tissues such as the brain. Previous histologic studies have shown that abnormal retinal oxygenation is involved in the pathogenesis of retinal diseases such as diabetic retinopathy,[2,3] retinal vein occlusion,[4,5] and glaucoma.[6,7] However, simple, noninvasive methods to measure retinal oxygen saturation levels in vivo were not previously available. Retinal oximetry is a noninvasive method that allows in vivo measurements of retinal oxygen saturation, based on the principle of differential light absorbance of oxygenated and deoxygenated blood at specific wavelengths of light.[8]

There have been an increasing number of studies using this technique with reports of variation in retinal vessel oxygen saturation measurements in eyes with diabetic retinopathy,[9,10] central retinal vein occlusion,[11,12] and possibly glaucoma.[13] For example, higher oxygen saturation in retinal arterioles and venules has been observed in eyes with diabetic retinopathy,[9,10] lower oxygen saturation in retinal venules has been observed in eyes with central retinal vein occlusion,[12] and severe glaucomatous damage has been associated with increased oxygen saturation in retinal venules.[14] These findings provide evidence of disruption of distribution of oxygen in diseased eyes.[9,12]

Although these data indicate usefulness in assessing retinal vessel oxygen saturation for different retinal diseases, the reliability of such measurement method has not been evaluated in detail. Palsson et al.[15] have briefly reported the intravisit repeatability of retinal oximetry, based on standard deviation of means from repeated measurements in the same visit, but there have been no additional studies. Furthermore, major systemic factors that may influence these measurements, such as blood pressure and body mass index, have not been investigated. Knowing the variability and influenced systemic factors of retinal vessel oximetry measurements is crucial before retinal oximetry can be considered for clinical use.

The purpose of our study was to assess the reliability of retinal vessel oximetry measurement by using the retinal oximeter and to investigate the major systemic determinants of retinal vessel oximetry measurements in normal healthy eyes.

Methods

Study Population

This was a cross-sectional study conducted from December 2011 to January 2013. This study was conducted with the

Table 1. Criteria for Choosing Retinal Vessel Segments for Measurement of Retinal Oximetry With the Oxymap T1 Retinal Oximeter

Retinal Vessel Selection	Criteria
Start vessel selection	147 pixels from disc margin (1 disc diameter from disc margin)
End vessel selection	200 pixels from the margin of no-measurement zone
Retinal vessel width	Minimum 8 pixels (1 pixel is approximately 9.25 μm[20])
Retinal vessel length	Minimum 100 pixels
Branching	Measure parent branch. If the parent branch is less than 50 pixels in length, daughter branches are also measured
Crossing	Choose distal segment from optic disc. If segment is less than 100 pixels in length, measure proximal segment

primary aim to investigate the reliability and reproducibility of measurements of retinal vascular structural and functional changes by using different ocular imaging modalities. This study included participants recruited from a community-based clinic located in the central district of Singapore. Patients from the clinic, older than 40 years, without a history of stroke or heart disease were invited to participate in the study. Patients with presence of maculopathy, glaucoma, or any forms of retinopathy were excluded from this study. This study was conducted by following the tenets of the Declaration of Helsinki and was approved by the SingHealth Institution Review Board. Written informed consent was obtained from each participant.

Retinal Oxygen Saturation Imaging and Measurement

Retinal oximetry imaging was performed after pupil dilation with 1% tropicamide by using the Oxymap T1 Retinal Oximeter (Oxymap, Reykjavik, Iceland) that is based on a fundus camera (Canon CR6-45NM; Canon, Inc., Tokyo, Japan) that is coupled with a beam splitter (MultiSpec Patho-Imager; Optical Insights, Tucson, AZ, USA) and a digital camera (SBIG ST-7E; Santa Barbara Instrument Group, Santa Barbara, CA, USA). Retinal oximetry procedures have been previously reported in detail.[16–18] In brief, two fundus images of the same area of the retina are simultaneously taken with two different wavelengths of light, 570 and 600 nm. At the oxygen-insensitive wavelength 570 nm the arterioles and venules are similarly dark, whereas at 600 nm the light absorbance decreases with increased oxygen saturation and arterioles appear much brighter than venules. Oxymap uses special built-in software to calculate the optical density.[8] The optical density ratio was calculated from optical density at the two wavelengths[16] by the Oxymap built-in software.

Retinal oximetry images were processed by using a built-in software (Version 2.3.1, Oxymap Analyzer; Oxymap). Measurements were performed by following a standardized protocol.[19] The optic disc–centered image was used for analysis. In each image, oximetry measurements were taken in all retinal arterioles and venules measuring more than 6 pixels (9.25 μm[20]) in vessel width in the measurement zone (Fig.). Vessel segments were selected by the user in a standardized manner (Table 1). The vessels were named according to the main branch and quadrant at which vessel was found. The quadrant was identified by the end point of the vessel. After detailed vessel segment selection, the Oxymap Analyzer software automatically measures the oximetry levels within each selected vessel. Vessel selection was done 1 disc diameter away from the disc margin to avoid uneven retinal background reflections near the optic disc margin (e.g., possibly caused by peripapillary atrophy nerve fiber reflections, retinal and choroidal pigmentation).

Intra- and Intergrader Reliability

Two trained graders, masked to subjects' characteristics, independently measured the 50 retinal oximetry images from 50 subjects to assess intergrader reliability. In addition, one grader repeated the measurements after 1 week to assess intragrader reliability.

Intravisit Repeatability

Intravisit repeatability was assessed by measuring oximetry levels of two different oximetry images (of the same eye) taken on the same visit. Twenty retinal oximeter image pairs from 20 subjects were assessed by one grader.

Overall and Quadrant Retinal Vessel Oximetry Measurements and A-V Difference

For the analyses of quadrant retinal arteriolar and venular oximetry measurements, vessels were categorized into the quadrants by the end point of the vessel: superior–temporal or superior–nasal, inferior–temporal, inferior–nasal, and also vessel types (i.e., arterioles or venules).

The Oxymap software detects vessels and calculates arterial and venular widths. The Oxymap analyzer software then takes into account the oximetry measurement variability that may arise owing to variations in vessel width via their correction formula.[8,21] The effect of vessel diameter on retinal oximetry measurements is hence being compensated for. Overall/quadrant retinal oximetry levels were calculated as the sum of each retinal oximetry measurement multiplied by the diameter of each vessel in the fourth power, further divided by the sum of diameter of each vessel in the fourth power.[21,22] If, for example, four venules were measured in an eye and each has measured saturation S and diameter D, the mean saturation becomes

$$\frac{(S_1 \times D_1^4) + (S_2 \times D_2^4) + (S_3 \times D_3^4) + (S_4 \times D_4^4)}{D_1^4 + D_2^4 + D_3^4 + D_4^4}.$$

The A-V difference was calculated as the difference between retinal arteriolar oximetry level and retinal venular oximetry level for each eye, an average of A-V differences was then calculated.[23]

Other Measurements

Medical history was obtained by using a standardized questionnaire administered by trained personnel. Age was defined as the age at the time of clinic examination. Height was measured in centimeters by using a wall-mounted measuring tape and weight was measured in kilograms by using a digital scale. Body mass index (BMI) is calculated as ratio of body weight (measured in kilograms) divided by the square of the body height (measured in meters). Diabetes was defined as a self-reported physician-diagnosed history of diabetes. Hypercholesterolemia was defined as a self-reported physician-diagnosed history of high cholesterol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by using a digital automatic blood pressure monitor (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies, Inc., Milwaukee, WI, USA), after the participant was seated for at least 5 minutes. Blood pressure

Table 2. Baseline Characteristic of the Study Cohort, Stratified by Sex

Characteristics	Male, $n = 45$ Mean (SD)/N, %	Female, $n = 73$ Mean (SD)/N, %	P Value
Age, y	54.69 (6.86)	52.93 (6.33)	0.158
Systolic blood pressure, mm Hg	135.04 (16.11)	122.08 (17.94)	<0.001
Diastolic blood pressure, mm Hg	83.44 (11.00)	71.40 (9.30)	<0.001
Central aortic systolic pressure, mm Hg	119.09 (16.24)	115.93 (16.79)	0.314
Body mass index, kg/m^2	23.31 (4.34)	23.64 (3.63)	0.657
Hypertension, yes	30 (66.70)	26 (35.60)	<0.001
Diabetes, yes	6 (13.30)	4 (5.50)	0.137
Hypercholesterolemia, yes	16 (35.6)	20 (27.4)	0.350

P value for differences between male and female participants by *t*-test or χ^2 test as appropriate.

was measured twice, 5 minutes apart. A third measurement was made if the SBP differed by more than 10 mm Hg or the DBP by more than 5 mm Hg. The mean between the two closest readings was then taken as the blood pressure for that individual. Hypertension was defined as SBP of at least 140 mm Hg or DBP of at least 90 mm Hg, a self-reported physician-diagnosed history of hypertension, or use of antihypertensive medication. Central aortic systolic pressure (CASP) was measured with a noninvasive wristwatch device (BPro, HealthStats, Singapore).

Table 3. Intra- and Intergrader Reliability and Intravisit Repeatability of Retinal Oximetry Measurements

Retinal Oximetry Measurements	ICC	95% CI
Intragrader		
SaO_2		
Superior–temporal	0.93	0.74–0.98
Superior–nasal	0.94	0.79–0.99
Inferior–temporal	0.99	0.98–0.99
Inferior–nasal	0.98	0.94–0.99
SvO_2		
Superior–temporal	0.99	0.96–0.99
Superior–nasal	0.99	0.97–0.99
Inferior–temporal	0.89	0.63–0.97
Inferior–nasal	0.94	0.71–0.99
Intergrader		
SaO_2		
Superior–temporal	0.94	0.81–0.98
Superior–nasal	0.77	0.69–0.89
Inferior–temporal	0.87	0.21–0.97
Inferior–nasal	0.94	0.90–0.98
SvO_2		
Superior–temporal	0.78	0.64–0.89
Superior–nasal	0.92	0.89–0.97
Inferior–temporal	0.94	0.87–0.97
Inferior–nasal	0.80	0.33–0.95
Intravisit		
SaO_2		
Superior–temporal	0.88	0.72–0.95
Superior–nasal	0.89	0.77–0.93
Inferior–temporal	0.96	0.51–0.98
Inferior–nasal	0.94	0.45–0.98
SvO_2		
Superior–temporal	0.92	0.52–0.99
Superior–nasal	0.85	0.71–0.95
Inferior–temporal	0.89	0.26–0.96
Inferior–nasal	0.95	0.25–0.97

SaO_2, retinal arteriolar oximetry measurement; SvO_2, retinal venular oximetry measurement.

Statistical Analysis

We selected one eye from each participant randomly for final analysis. All statistical analyses were performed by using SPSS statistics version 17.0 (SPSS, Inc., Chicago, IL, USA).

Retinal vessel oximetry measurements were analyzed as continuous variables. We used intraclass correlation coefficients (ICCs) to evaluate the intra- and intergrader reliability and intravisit repeatability. Univariable linear regression models were performed to determine the association between systemic factors and retinal vessel oximetry levels of arterioles and venules (dependent variables).

Results

We excluded eight (5.88%) subjects owing to poor image quality or lack of fundus photography or retinal oximetry images. We included 118 subjects in the final analysis. Table 2 compares the baseline characteristics of the study population between male and female.

Table 4. Distributions of Retinal Oximetry Levels in Normal Eyes

Retinal Oximetry	Mean (SD)	Interquartile Range
SaO_2, %		
Overall	93.64 (6.9)	92.18–95.73
ST	92.14 (12.0)	90.07–94.22
SN	96.15 (8.6)	94.66–97.65
IN	95.43 (11.5)	93.41–97.44
IT	90.11 (10.1)	88.28–91.94
SvO_2, %		
Overall	54.22 (6.9)	52.75–55.68
ST	55.82 (8.2)	54.40–57.23
SN	54.47 (10.0)	52.72–56.21
IN	55.13 (9.8)	53.40–56.85
IT	46.47 (11.2)	44.41–48.52
A-V difference in SO_2, %		
Overall	39.43 (8.9)	37.53–41.33
ST	33.62 (9.0)	31.72–35.52
SN	40.7 (9.7)	38.66–42.75
IN	40.2 (14.6)	37.11–43.29
IT	43.2 (13.9)	40.29–46.14

SO_2, retinal oximetry measurement; SD, standard deviation.

Table 5. Association Between Systemic Factors and Retinal Oximetry Measurement (Univariable Analysis)

	Overall	ST	SN	IN	IT
			SaO_2 (95% CI)		
Systemic					
Age, y; every 10-y increase	1.61 (−0.46 to 3.67)	0.83 (−2.31 to 3.97)	1.56 (−0.73 to 3.84)	0.85 (−2.25 to 3.94)	3.37 (−0.58 to 6.16)
Sex	−0.96 (−3.75 to 1.83)	−1.34 (−5.65 to 2.96)	0.55 (−2.59 to 3.69)	−0.91 (−5.16 to 3.35)	−2.11 (−5.94 to 1.73)
SBP, mm Hg	0.07 (−0.004 to 0.15)	0.06 (−0.06 to 0.18)	0.05 (−0.03 to 0.14)	0.1 (−0.02 to 0.21)	0.1 (−0.01 to 0.21)
DBP, mm Hg	0.07 (−0.06 to 0.20)	0.06 (−0.12 to 0.25)	0.04 (−0.09 to 0.17)	0.11 (−0.07 to 0.29)	0.11 (−0.07 to 0.29)
CASP, mm Hg	0.03 (−0.06 to 0.11)	0.08 (−0.05 to 0.21)	−0.05 (−1.15 to 0.04)	−0.004 (−0.13 to 0.12)	0.04 (−0.07 to 0.15)
BMI, kg/m^2	0.06 (−0.24 to 0.36)	−0.03 (−0.51 to 0.45)	0.14 (−0.20 to 0.49)	0.11 (−0.36 to 0.57)	−0.003 (−0.42 to 0.41)
Hypertension	1.73 (−0.94 to 4.41)	0.21 (−3.96 to 4.38)	0.61 (−2.39 to 3.61)	4.79 (−0.82 to 8.75)	3.44 (−0.21 to 7.08)
Diabetes	2.6 (−2.26 to 7.47)	3.74 (−3.16 to 10.65)	0.58 (−4.42 to 5.59)	1.86 (−5.09 to 8.80)	−3.56 (−10.18 to 3.06)
Hypercholesterolemia	0.24 (−2.69 to 3.16)	−3.32 (−7.86 to 1.22)	0.001 (−3.30 to 3.30)	2.41 (−2.04 to 6.85)	−1.15 (−5.15 to 2.86)
			SvO_2 (95% CI)		
Systemic					
Age, y; every 10-y increase	−2.61 (−4.92 to −0.29)*	−1.75 (−3.89 to 0.38)	−2.52 (−5.13 to 0.10)	−3.53 (−6.07 to −0.99)*	−2.86 (−5.90 to 0.18)
Sex	0.98 (−2.33 to 4.28)	1.07 (−1.88 to 4.03)	2.52 (−1.10 to 6.13)	−0.59 (−4.17 to 2.99)	0.63 (−3.37 to 5.37)
SBP, mm Hg	−0.04 (−0.13 to 0.05)	0.01 (−0.07 to 0.09)	−0.001 (−0.1 to 0.10)	−0.04 (−0.14 to 0.06)	−0.05 (−0.17 to 0.07)
DBP, mm Hg	−0.05 (−0.19 to 0.09)	−0.02 (−0.14 to 0.11)	0.01 (−0.14 to 0.17)	0.001 (−0.15 to 0.16)	−0.09 (−0.27 to 0.09)
CASP, mm Hg	−0.05 (−0.14 to 0.05)	−0.03 (−0.12 to 0.05)	−0.11 (−0.21 to 0.001)	−0.1 (−0.21 to 0.01)	−0.08 (−0.21 to 0.04)
BMI	0.08 (−0.29 to 0.44)	0.04 (−0.29 to 0.37)	0.08 (−0.32 to 0.49)	0.09 (−0.31 to 0.48)	0.12 (−0.36 to 0.59)
Hypertension, yes	−1.25 (−4.44 to 1.94)	−0.71 (−3.54 to 2.13)	0.42 (−3.09 to 3.92)	−1.61 (−5.06 to 1.85)	−0.91 (−5.04 to 3.22)
Diabetes, yes	−0.17 (−5.56 to 5.23)	−1.05 (−5.79 to 3.69)	1.49 (−4.55 to 7.53)	3.06 (−2.61 to 8.73)	−2.23 (−9.27 to 4.80)
Hypercholesterolemia	−1.95 (−5.41 to 1.51)	−1.87 (−4.95 to 1.21)	1.12 (−2.73 to 4.96)	1.16 (−2.65 to 4.96)	−3.81 (−8.29 to 0.68)

* P value < 0.05.

Intra- and intergrader reliability and intravisit repeatability assessment are shown in Table 3. The ICC values ranged from 0.89 to 0.99 for intragrader reliability, 0.77 to 0.94 for intergrader reliability; and 0.85 to 0.96 for intravisit repeatability.

Table 4 shows the overall and quadrant retinal oximetry measurements and A-V difference in oximetry values. The overall retinal arteriolar oximetry value was 93.64% (SD: 6.9%; interquartile range, 92.18%–95.73%) and the overall retinal venular oximetry value was 54.22% (SD: 6.9%; interquartile range, 52.75%–55.68%). The overall A-V difference was 39.43% (SD: 8.9%; interquartile range, 37.53%–41.33%).

Table 5 shows the univariable analyses of retinal oximetry measurements with systemic determinants. Older age was significantly associated with reduced retinal venular oximetry levels overall (β: −2.61%; 95% CI: −4.92 to −0.29) and at the inferior-nasal quadrant (β: −3.53%; 95% CI: −6.07 to −0.99). No associations were observed between retinal arteriolar oximetry measurements and any systemic factors.

Discussion

We provided new data on the reliability of retinal oximetry measurements by using the Oxymap Retinal Oximeter and a wide spectrum of systemic determinants of the measures in normal eyes. These findings are important for the interpretation of retinal oximetry measurement and for future investigation of retinal oximetry levels in diseased eyes.

First, we showed that retinal oximetry measurements were highly repeatable. Although a few studies have reported on the repeatability of retinal oximetry,[16,18] insufficient analysis and study methods mean that repeatability of retinal oximetry have not been assessed in detail. Palsson et al.[15] have determined the repeatability of retinal oximetry, based on standard deviation of means from repeated measurements in the same visit, while Geirsdottir et al.[16] have determined reliability of retinal oximetry by comparing retinal oximetry measurements between right and the left eyes. In addition, the image acquisition and measurement protocol used by Palsson et al.[15] has also not been specified.

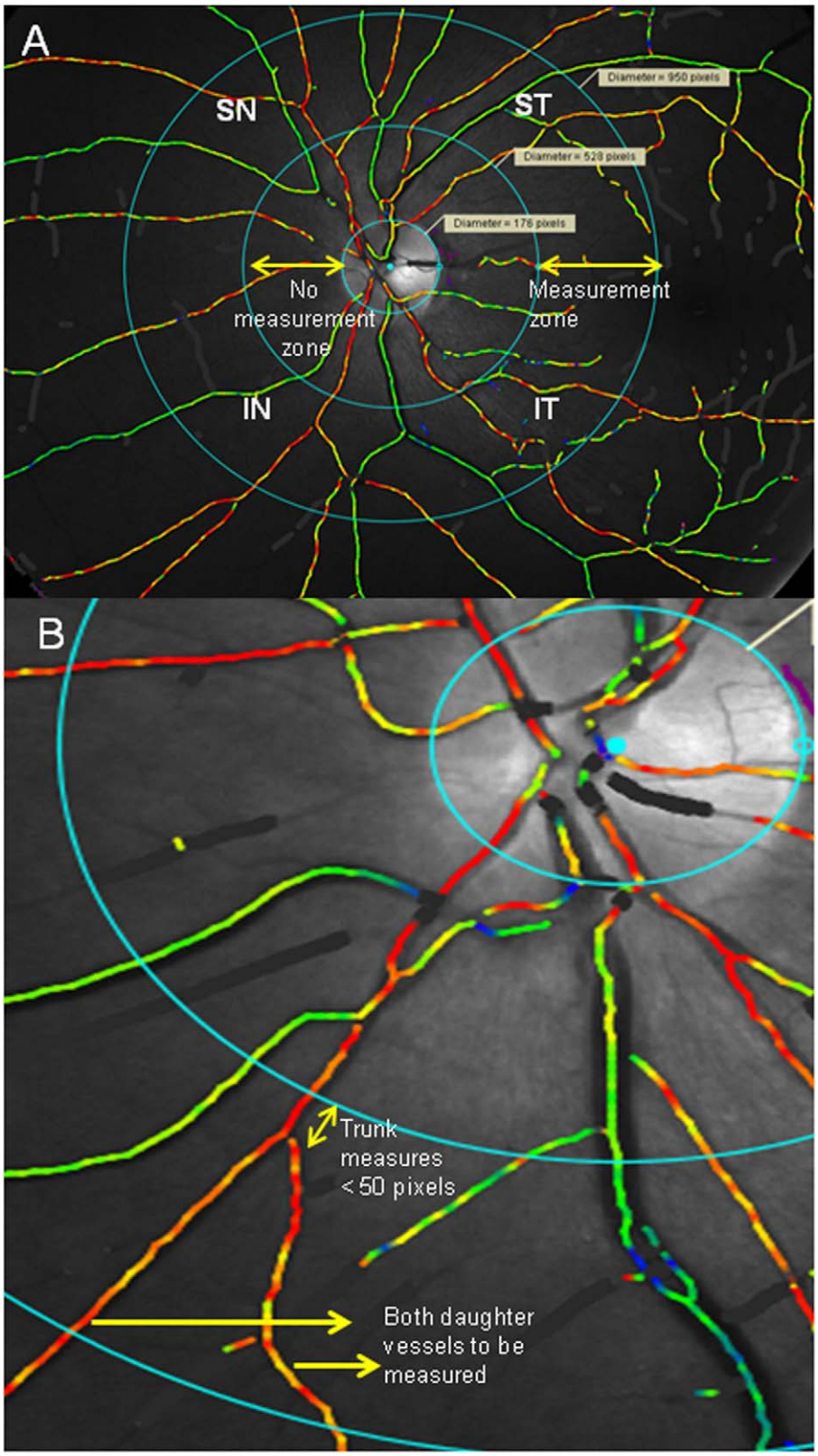

FIGURE. (A) Disc-centered image for retinal vessel oximetry measurement. All arterioles and venules within the measurement zone are measured. The locations of the vessels are marked, and categorized into superior–nasal (SN), superior–temporal (ST), inferior–temporal (IT), and inferior–nasal (IN) groups. No-measurement zone is defined as 1 disc diameter from the disc margin; measurement zone is measured 200 pixels starting from the border of the no-measurement zone. (B) If the length of the vessel trunk is less than 50 pixels, the daughter vessels will be then measured instead.

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In our study, using standardized image acquisition and measurement protocol, we demonstrated good intra- and intergrader reliability as well as intravisit repeatability of retinal oximetry measurements. The ICC analysis in the current study better affirms the reliability and repeatability of retinal oximetry measurements, compared to previous studies. Variations in image acquisition protocol have been suggested to have an impact on retinal vessel oximetry measurements. For example, Heitmar and Cubbidge[24] have reported that a high flash intensity leads to higher than normal oximetry measurements and Palsson et al.[15] also have found that inconsistent directions of gaze affect measured oximetry levels. Thus, we would highlight the use of standardized protocol for image acquisition and measurement to obtain high reproducible retinal vessel oximetry measurements.

Second, we found that age was inversely associated with overall and inferior–nasal retinal venular oximetry levels. Previous studies have reported conflicting results on the associations between retinal oximetry levels and age. Similar to our findings, Geirsdottir et al.[16] have reported significant inverse association between age and retinal venular oximetry levels (older age is associated with reduced retinal venular oximetry levels). Jani et al.[8] have found a significant inverse association not only between age and retinal venular oximetry levels but also with retinal arteriolar oximetry levels. On the other hand, Michelson and Scibor[25] have not observed any associations between age and retinal arteriolar and venular oximetry levels. Separately, it has also been reported that arteriolar oxygen saturation measured by using pulse oximetry decreases with increasing age.[26] It has been postulated that this inverse association between oxygen saturation and age could be caused by age-related functional and structural changes at the cellular level.[27] As such we believe further studies are required to elucidate the effect of aging on retinal vessel oximetry levels.

Distributions of retinal oximetry levels in normal eyes in this study were similar to those reported in some previous studies,[8,9,16] which also used Oxymap Retinal Oximeter. Our study measured average retinal arteriolar oximetry levels to be 93.64% (SD: 6.89%; interquartile range, 92.18%–95.73%) and average retinal venular oximetry levels to be 54.22% (SD: 6.91%; interquartile range, 52.75%–55.68%; Table 4) in Asian eyes. In comparison, Hardarson and Stefansson[12] have measured retinal arteriolar oximetry levels to be 99% ± 6% and retinal venular oximetry levels to be 65% ± 6%, while Hammer et al.[28] have measured retinal arteriolar oximetry levels to be 98% ± 10.1% and retinal venular oximetry levels to be 65% ± 11.7% in Caucasian eyes. Overall retinal oximetry levels have been found not to vary across ethnicity.[8] Furthermore, it has been suggested that the difference could be due to the use of different oximeter and measurement protocols.[8] Hardarson and Stefansson[12] have used Oxymap Retinal oximeter (Oxymap ehf, Reykjavik, Iceland), while Hammer et al.[28] have used a retinal oximeter prototype. Additionally, the vessel diameter correction used by Hammer et al.[28] differs from that of our protocol. The measurement protocol used has also not been specified by Hardarson and Stefansson[12] and only briefly by Hammer et al.[28] As such, for accurate comparisons of average retinal oximetry measurement across populations, retinal oximeter and measurement protocol software should be standardized.

An observable disparity in retinal arterial oximetry levels between the quadrants has previously been reported by Jani et al.,[8] Palsson et al.,[15] and Geirsdottir et al.[16] In our study, we observed that retinal oximetry measurements were evenly distributed across the four quadrants (superior–temporal, superior–nasal, inferior–temporal, and inferior–nasal). Our measurements were done further away from the optic disc margin (1 disc diameter) as compared to previous studies. This was to avoid the uneven retinal background reflections (possibly caused by retinal and choroidal pigmentation or retinal nerve fiber myelination) near the optic disc margin, which may be a cause of the uneven spread in retinal oximetry levels across the quadrants. Hence, it is possible that uneven spread of data across the quadrants reported in previous studies may be a result of uneven retinal background reflections near the optic disc and possibly not a result of physiological mechanism.

The strengths of our study included standardized clinical examination and the image acquisition and oximetry measurement protocols used. Nevertheless, this study had a few limitations. First, the causative relationship between systemic determinants with retinal oximetry measurement could not be definitively assessed owing to the cross-sectional nature of our data. Second, as systemic oxygen saturation was not measured, we could not validate the agreement between retinal oximetry measurements and systemic oxygen saturation levels. Third, the association between fundus pigmentation and retinal oximetry levels could not be investigated in our study. Such possible factor should be controlled for (e.g., iris color) in the further development of oximetry technology.

In conclusion, retinal oximetry is repeatable with a standardized image acquisition and measurement protocol. Age is the main factor that influences retinal vessel oximetry measurements. These findings are important for future investigation of retinal vessel oximetry levels between diseased and nondiseased eyes.

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