Diurnal Variations in the Morphology of Schlemm’s Canal and Intraocular Pressure in Healthy Chinese: An SS-OCT Study

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PURPOSE. To characterize the diurnal variations in the dimensions of the Schlemm’s canal (SC) and its association with intraocular pressure (IOP) using swept-source optical coherence tomography (SS-OCT).

METHODS. The temporal, nasal, inferior, and superior limbus of 102 eyes of 51 healthy subjects were imaged in vivo by SS-COT at 5 time points of 8 AM, 11 AM, 2 PM, 5 PM, and 8 PM. IOP was measured at the same time by Goldmann applanation tonometry (GAT). The diameter and the cross-sectional area of the SC were measured in ImageJ. The associations between changes in the SC parameters, IOP, and other biometric parameters were determined using a general estimating equations model. The temporal and inferior limbus of 94 eyes of 47 healthy subjects were also imaged before and during the Valsalva maneuver (VM) at 8 PM.

RESULTS. Mean IOPs at different time points were 13.37, 12.89, 11.9, 12.02, and 12.36 mm Hg. Of all four quadrants, the detectable rate of SC was highest in the superior quadrant (85.3%) and lowest in the inferior quadrant (75.5%). We found that changes in the SC area and diameter were negatively associated with IOP changes only in the inferior quadrant (P = 0.0046 and P = 0.0332, respectively), after adjusting for age, sex, eye, spherical equivalent, and axial length. The mean SC area and diameter during the VM were significantly higher than prior to the VM (P < 0.001).

CONCLUSIONS. The changes in the SC parameters were negatively associated with IOP changes only in the inferior quadrant. The VM could expand the SC in healthy subjects. Imaging of the SC may be a useful method to discover the reason why IOP fluctuates, and how SC changes morphologically during the daytime in the future.

Keywords: Schlemm’s canal, intraocular pressure, Valsalva maneuver

The rate of ocular aqueous humor (AH) drainage can affect intraocular pressure (IOP).1 In normal people, the aqueous humor is mainly drained through trabecular meshwork into the Schlemm’s canal (SC), and then it flows into collector canals and episcleral veins. Aging, inflammation, and other immune factors could change the structural component in the outflow pathway of the AH, leading to imbalance in AH dynamics.2-4 Blockage in any of the structures above will lead to the obstruction of AH drainage, causing ocular hypertension.5 Elevated IOP is an important risk factor for glaucoma and optic nerve damage.6

Before the 21st century, the observation of SC in vivo was limited by imaging techniques. With the invention of high-resolution optical coherence tomography (OCT), SC imaging in vivo became possible. Previous studies using OCT demonstrated that the SC area in patients with primary open angle glaucoma (POAG) and pseudoexfoliation glaucoma was decreased compared to normal people.7-9 In addition, the morphology of SC can be affected by the application of prostaglandins and surgery. A study by Chen et al.10 showed that the SC area expanded following the use of travoprost in healthy subjects. Similarly, expansion of SC was also detected after trabeculectomy and proved to be associated with the extent of IOP reduction.11 Yet, we still know little about the physiology of SC in normal people. As there is IOP fluctuation throughout the whole day,12-14 it is not known if there will be “morphological fluctuation” of the SC. Moreover, are there other factors, such as autonomic nervous activity, influencing the morphology of SC in addition to IOP?

The Valsalva maneuver (VM) is a forced exhalation against a closed airway, triggering a series of physiologic changes.15 Ocular structures are also affected. Elevated IOP, narrowed anterior chamber width, and increased lens vault, are observed during the VM,16,17 but the effect of VM on SC has not been studied. As the VM can affect anterior chamber structures through the autonomic nervous system, it is also possible that the morphology of SC could be changed during the VM.

To discover whether diurnal IOP variations affect SC morphology, we performed a prospective study in healthy Chinese subjects. We used swept-source OCT (SS-OCT) to image the SC at different time points in the daytime. We also imaged the SC during the VM. Our aim was to ascertain the physiologic factors associated with morphologic changes in SC in normal people.
METHODS

Subject Recruitment

We recruited study participants from the Sun Yat-sen University Medical School, Zhongshan Ophthalmic Center of Sun Yat-sen University, and nearby communities in Guangzhou, China. All patients signed a written informed consent form before entering the study. The study was approved by the Ethical Review Committee of the Zhongshan Ophthalmic Center and was conducted in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. We enrolled subjects aged between 18 and 45 years with a normal visual-field test. All participants had IOP less than or equal to 21 mm Hg. Subjects were excluded if they had: (1) systemic diseases (e.g., hypertension, diabetes mellitus, and severe cardiopulmonary insufficiency); (2) a history of use of topical ocular medications, such as corticosteroid; (3) current other ocular diseases (e.g., conjunctivitis, cataract, fundus diseases); (4) previous or current signs of uveitis; (5) previous ocular surgery; (6) high myopia or hyperopia (spherical equivalent [SE] refractive error greater than –6 or +3 diopters [D]); (7) clinically relevant opacities of the optic media (that may influence the observation of ocular fundus, and could be the signs of previous ocular inflammation); and (8) poor compliance in performing the VM correctly.

VM Training

Each participant received VM training before they entered the study. During the VM, we asked each participant to first take a deep breath and then blow forcefully against his hand and closed glottis, while squeezing his nose with his index finger and thumb. The VM was sustained during the examinations by maintaining the expiratory pressure against the hand and the glottis. To ensure participants’ compliance with performing the VM, we used an electrocardiograph monitor to assess their heart rate changes. The examiner took images of the anterior chamber angle after VM was performed for at least 15 seconds. The VMs were performed continuously until the images acquisition processes were finished, and then the participants started to breathe. Between two VMs of each eye, participants were given a short break of 5 minutes. Each participant was given a 15-minute rest between two examinations on different eyes.

Ocular Examinations

Before enrollment, all subjects underwent detailed ocular examinations, including best-corrected visual acuity, slit-lamp examination, fundus examination, and axial length (AL) measurements by optical biometer (IOL-Master; Carl Zeiss Meditec, La Jolla, CA, USA). We chose the Goldmann applanation tonometer (GAT) to measure IOP at five different time points. A refractive error examination was performed using an autorefractometer (KR-8900, version 1.07; Topcon Corp., Tokyo, Japan). After baseline AL and refractive error information was obtained, each participant received the GAT and OCT examinations in a sitting position at five time points (8 AM, 11 AM, 2 PM, 5 PM, and 8 PM) during the same day. The VM was performed after obtaining IOP and OCT images at 8 PM, and during the VM we repeated OCT examination on the anterior chamber angle.

SS-OCT Imaging

We obtained images of the anterior chamber angle by CASIA-OCT (SS-1000; Tomey, Nagoya, Japan). The CASIA-OCT system uses a tunable laser with a center wavelength of 1310 nm as a light source. A three-dimensional imaging scan procedure was performed with a 6 × 6 mm raster scan centered on the corneoscleral limbus at the superior, nasal, inferior, and temporal sides, which was composed of 256 A-scans (a total of 65,536 axial scans/volume). The participants received OCT examinations in a sitting position, and the same scan procedure was repeated at the five time points described above. Participants were asked to stare at a fixation point when scanning. To avoid the lid artifact, participants were instructed to pull down the lower lid or upper lid to expose the superior or inferior limbus. To ensure the exact scanning cross-section of SC at different time points using SS-OCT, we refer to the crypts and furrows of the iris as the landmarks (Fig. 5). Images with artifacts caused by blinking and eye movements were not included in the data analysis.

SC Measurements

The CASIA-OCT images of SC were first enhanced with the built-in adaptive compensation algorithm (available in the commercial CASIA-OCT® device) to make the boundary of SC clearer. Measurements of the SC diameter and area were completed by ImageJ (http://imagej.nih.gov/ij/; provided by the National Institutes of Health, Bethesda, MD, USA). The SC diameter was defined as the mean value of three measurements of the sagittal axial length of the thin, black, lucent space on the CASIA-OCT images. The SC area was drawn freehand and depicted the area surrounded by the outline of SC. The mean of the superior, nasal, inferior, and temporal SC parameters was included in the analysis.
imaging and the timing of CASIA-OCT in regard to VM (before or during VM). The values for the SC parameters were calculated as mean of both examiners’ (KG, FL) measurements.

Statistical Analysis

Statistical analyses were performed using statistical software (Stata 14.0; StataCorp LLC, College Station, TX, USA). The means and SDs were calculated for all the measured parameters. Paired t-tests were used to detect the differences in the parameters between the baseline and during the VM. Repeated measures analysis of variance models were performed to detect the variations of SC parameters during daytime. We captured another SC image in temporal quadrant of 16 eyes in an interval of 1 week by the same observer (KG). To investigate the differences between two measurements of SC parameters in different image acquisitions, we performed a Bland-Altman (BA) analysis. Univariate and multivariate linear regression was used to determine the relationship between the changes in the parameters of SC, IOP, and other biometric parameters. Multivariate linear regression was performed using the generalized estimating equations (GEEs) model, with 95% confidence intervals (CIs), taking into account the correlation between the measurements from two eyes. A P-value of less than 0.05 was considered statistically significant.

RESULTS

We included 102 eyes of 51 normal subjects in this study. Baseline and demographic characteristics are shown in Table 1. Of the participants, 21 were male and 30 were female. The mean age of all the participants was 23.24 ± 2.52 years. Table 2 summarizes the fluctuations in the IOP and mean age of all the participants was 23.24 ± 2.52 years. Table 2 summarizes the fluctuations in the IOP and other biometric parameters. Paired t-tests were used to detect the differences in the parameters between the baseline and during the VM. Repeated measures analysis of variance models were performed to detect the variations of SC parameters during daytime. We captured another SC image in temporal quadrant of 16 eyes in an interval of 1 week by the same observer (KG).

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VM. The IOP and SC parameters before and during the VM were summarized in Table 4. The mean IOP before and during the VM was 14.26 ± 2.21 and 15.39 ± 2.66 mm Hg, respectively. The mean SC areas in the inferior quadrant before and during the VM were 230.26 ± 94.16 and 382.04 ± 128.25 pixels (P < 0.001), and in the temporal quadrant were 242.05 ± 86.69 and 390.83 ± 125.28 pixels (P < 0.001). The mean SC diameters in the inferior quadrant before and during the VM were 6.74 ± 1.15 and 8.00 ± 1.4 pixels (P < 0.001), and in the temporal quadrant were 6.83 ± 1.18 and 8.17 ± 1.44 pixels (P < 0.001). A paired t-test of the IOP and SC parameters before and during the VM showed statistical significance in the inferior and temporal quadrants (P < 0.001). An association study of the changes in the SC area, SC diameter, and other demographic parameters used the GEEs model (Supplementary Table S5). The results showed that age (P = 0.046) was significantly and positively related to changes in the SC area only in the inferior quadrant. However, SC parameters and other variables were not significantly correlated (P > 0.05).

**DISCUSSION**

In the present study, we imaged the dynamic changes in SC during the daytime using SS-OCT with high-quality images. We found that only the SC area and diameter in the inferior quadrant was negatively associated with IOP fluctuations in healthy subjects. During the five time points during the daytime, the fluctuations in IOP were not significant. Compared with the baseline, IOP, the SC area, and the SC diameter all increased significantly during the VM in our study. To the best of our knowledge, this is the first study to investigate the relationship between the SC parameters and IOP of several time points of the same subjects during the daytime.

IOP fluctuates throughout the day in both healthy subjects and patients with glaucoma.6,20–24 In our study, we found that after adjusting for age, sex, eye, SE, and AL, only the area and diameter of the SC at the inferior quadrant were negatively associated with changes in IOP (P < 0.05). We speculated that the SC of the inferior part was more easily affected by the gravity and the fluid dynamics of the AH. The other parts of SC, however, were not as easily influenced by changes in the AH, especially in the superior part. The outflow of aqueous humor has its segmental patterns around the SC circumference, and the majority of AH outflow is likely through the inferior collector channels.25,26 Overall, the distal flow structures (e.g., collector channels and episcleral veins) may be have more importance than previously believed. Our results showed that the SC parameters of inferior quadrant were smaller than the other three quadrants. We hypothesized that the effect of gravity and the pressure of AH were more likely to decrease SC

**FIGURE 3.** Mean SC area at five different time points. The SC area value at five different time points of four quadrants was defined as all recruited subjects at each time point.

**FIGURE 4.** Mean SC diameter at five different time points. The SC diameter value at five different time points of four quadrants was defined as all recruited subjects at each time point.
Dynamic Observation of Schlemm’s Canal

Inferior diameter, pixels 6.74 1.15 8.00 1.40

Dependent Variables $\beta$ SD Z $P^*$

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<th>Area</th>
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<td>Superior</td>
<td>9.45</td>
<td>7.4831</td>
<td>1.26</td>
</tr>
<tr>
<td>Nasal</td>
<td>22.5016</td>
<td>11.8088</td>
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<tr>
<td>Superior</td>
<td>4.7039</td>
<td>12.1284</td>
<td>0.39</td>
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Factors with statistical significance are shown in bold type ($P < 0.05$).

* Acquired after adjusting age, sex, eye, SE and AL using GEEs model.

Previously collected parameters of inferior quadrant. Besides, it was suitable to maintain the stable outflow of AH at inferior quadrant of the eye. Otherwise, the larger SC parameters combined with larger collector channels were likely to drainage excessive AH resulting in lower IOP of the eye.

To avoid the compressive and physiologic impact on SC and other anterior ocular parameters using ultrasound biomicroscopy, we chose the SS-OCT in this study. SC images detected using CASIA-OCT had good quality, and the parameters of SC measurements had excellent intraobserver and interobserver agreement. CASIA-OCT may play an important role as clinical assessment equipment to evaluate the outcome of SC targeting surgery in the future.

SC is a part of the traditional AH outflow pathway, which could be affected by age, IOP, exercise, sympathetic nerve activation, and pharmacologic agents. Yan et al. demonstrated that aerobic exercise could cause the expansion of the TM and SC of healthy subjects by means of stimulation to the sympathetic nervous system, which eventually reduced the IOP. In our opinion, however, since the VM could activate the vagal nerve, which belongs to the parasympathetic nervous system, causing the SC area increase during the VM, we speculated that the SC might be affected by the sympathetic and parasympathetic nervous systems simultaneously.

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Conclusions

In this study, SC parameters can be noninvasively evaluated by SS-OCT. We discovered that IOP fluctuations were associated
with the parameters of the inferior quadrant of SC in healthy subjects. In addition, the VM can expand the SC in both temporal and inferior parts, which was correlated with IOP elevations. These results confirm that SC parameters may be factors that regulate the IOP through the AH turnover pathway.

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