Reduced Pulsatile Trabecular Meshwork Motion in Eyes With Primary Open Angle Glaucoma Using Phase-Sensitive Optical Coherence Tomography

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Received: February 4, 2020
Accepted: November 24, 2020
Published: December 16, 2020

Citation: Gao K, Song S, Johnstone MA, et al. Reduced pulsatile trabecular meshwork motion in eyes with primary open angle glaucoma using phase-sensitive optical coherence tomography. Invest Ophthalmol Vis Sci. 2020;61(14):21. https://doi.org/10.1167/iovs.61.14.21

Glaucoma

Glaucoma is the leading cause of irreversible blindness worldwide, which is characterized by progressive loss of retinal ganglion cells that results in corresponding visual field (VF) defects.1 POAG is the most prevalent form of glaucoma and elevated IOP is perhaps the most significant and modifiable risk factor.2–4 However, the cause of IOP elevation in POAG is less understood. IOP homeostasis depends on the normal function of the aqueous humor (AH) outflow system, consisting of the conventional and uveoscleral outflow pathways. Aqueous outflow facility, a measure of the TM. This study's findings are consistent with TM dysfunction in POAG eyes. Our evidence suggests that the measurement of pulsatile TM motion with PhS-OCT may help in characterizing outflow pathway abnormalities.

Keywords: trabecular meshwork, primary open angle glaucoma, phase-sensitive optical coherence tomography, pulsatile motion

PURPOSE. The purpose of this study was to investigate the difference in pulsatile trabecular meshwork (TM) motion between normal eyes with POAG using phase-sensitive optical coherence tomography (PhS-OCT).

METHODS. In this cross-sectional study, eight healthy subjects (16 eyes) and nine patients with POAG (18 eyes) were enrolled. A laboratory-based prototype PhS-OCT system was used to measure pulsatile TM motion. PhS-OCT images were analyzed to obtain parameters of pulsatile TM motion (i.e. maximum velocity [MV] and cumulative displacement [CDisp]). Outflow facility and ocular pulse amplitude were measured using pneumotonomography. Detection sensitivity was compared among various parameters by calculating the area under the receiver operating characteristic curves (AUCs).

RESULTS. A pulsatile TM motion waveform synchronous with digital pulse was observed using PhS-OCT in both healthy and POAG eyes. The mean MV in eyes with glaucoma was significantly lower than healthy eyes (P < 0.001). The mean CDisp in POAG eyes was also significantly lower than healthy eyes (P < 0.001). CDisp showed a significant correlation (r = 0.46; P = 0.0088) with ocular pulse amplitude in the study. Compared with the outflow facility, both the MV and CDisp were found to have a better discrimination of glaucoma (P < 0.001 and P = 0.0074, respectively).

CONCLUSIONS. Pulsatile TM motion was reduced in patients with POAG compared to healthy subjects. The underlying mechanism may be due to the altered tissue stiffness or other biomechanical properties of the TM in POAG eyes. Our evidence suggests that the measurement of pulsatile TM motion with PhS-OCT may help in characterizing outflow pathway abnormalities.

Keywords: trabecular meshwork, primary open angle glaucoma, phase-sensitive optical coherence tomography, pulsatile motion
pulsatile TM motion be effective in predicting the presence of glaucoma?

To address these questions, we conducted this cross-sectional study to investigate the difference in pulsatile TM motion between healthy patients and patients with POAG.

**METHODS**

**Subjects**

This cross-sectional study was conducted at the University of Washington Medicine Eye Institute between September 2018 and June 2019. This study was approved by the Ethical Review Committee of University of Washington (Seattle, WA, USA). This study adhered to the tenets of the Declaration of Helsinki and was conducted in compliance with the Health Insurance Portability and Accountability Act (HIPAA). Written informed consent was obtained from all subjects.

All subjects received comprehensive ocular examinations that included a review of their medical history, best-corrected visual acuity, a slit-lamp examination, and a stereoscopic optic disc examination with a 90-diopter lens. Blood pressure (BP) was measured using an automatic BP device (Welch Allyn Spot Vital Signs, version 2; Welch Allyn Inc., Skaneateles Falls, NY, USA). We defined the mean arterial pressure (MAP) as diastolic BP plus one third times (systolic BP minus diastolic BP). IOP was measured using a portable rebound tonometer (iCare, ic100; iCare, Helsinki, Finland).

Measurements of aqueous outflow facility and ocular pulse amplitude were obtained noninvasively by 2-minute tonography using a pneumotonometer per standard methods (Model 30; Reichert, Depew, NY, USA). The axial length (AL) and anterior chamber depth (ACD) were measured using partial coherence interferometry biometer (IOL Master, version 5.4; Carl Zeiss Meditec, La Jolla, CA, USA). POAG was defined as the presence of a normal open angle on gonioscopy, an untreated IOP of >21 mm Hg, and glaucomatous optic nerve damage with a corresponding vertical VF defect as confirmed by at least two reproducible VF tests. All patients with POAG received standard automated perimeter examination on a Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA), and only reliable tests were accepted (<20% fixation losses and false-positive and false-negative rates). Subjects were excluded if they: (1) had previous intraocular surgeries other than uncomplicated cataract surgery at least 6 months prior (e.g. laser trabeculoplasty or glaucoma surgery, etc.); (2) had other ocular diseases (e.g. uveitis, conjunctivitis, and fundus diseases); (3) had known cardiac arrhythmias; or (4) had myopia worse than a spherical equivalent of -4 diopters.

**PhS-OCT Examination**

An experienced investigator (K.G.) performed the PhS-OCT examinations in the same dimly lit room of stable illumination in the morning at approximately 10 AM. All participants were in a sitting position during PhS-OCT examinations. The laboratory PhS-OCT system prototype was comprised of three parts: a spectral domain OCT (SD-OCT) system, a digital pulsimeter, and an external controlling unit. The control unit was used to synchronize OCT data acquisition and cardiac signal recording, as described in our previous study. Briefly, the SD-OCT system used a superluminescent diode with a wavelength of 1310 nm as the light source with a spectral bandwidth of 100 nm, giving an axial resolution of approximately 5.5 μm in tissue. The lateral resolution of approximately 16 μm was determined by the objective lens (50 mm focal length) used to focus the sample beam into the sample. A repeated B-scan pattern covering 3.5 mm was performed and that was centered on the temporal limbus. For each imaging session, 2000 OCT B-scans (400 B-scan/s) were captured, lasting for a duration of 5 seconds, which approximately covers 5 cardiac cycles. Because the TM movement is the target of the measurement in this investigation, the system sensitivity is a more important metric of concern. The metric is a measure of the smallest displacement that can be measured under the intrinsic system noise floor. The system noise floor was measured at approximately 15 mrad using an ex vivo cornea tissue to simulate in vivo anterior segment tissue. The measurement translates to a minimal displacement of approximately 1.2 nm that the SD-OCT system can measure (assuming that the average refractive index of the tissue is 1.35). Correspondingly, given the system imaging speed of 400 fps (i.e. Δt = 2.5 ms), the minimum resolvable velocity for the current phase sensitive SD OCT system is approximately 0.48 μm/s. For detailed information on how to characterize the OCT system for dynamic displacement measurement, please refer to the paper by Wang et al. Another pertinent concern is the issue of involuntary movement of the patient during imaging, which must be removed during the data processing. In dealing with this, we used a proprietary technique in which a phase compensation strategy is used to remove the bulk tissue movement induced by involuntary patient motion in the measurements. If readers are interested, please refer to the papers by An et al. for more details of this strategy.

During imaging, consistent positioning was achieved as follows. Patients’ faces were oriented in a straight-ahead position. Patients then looked at an external fixation target without moving their head, placing the eye in an optimal position for the PhS-OCT to acquire images of the temporal limbus. An identical protocol was used for each participant. Three repeated scans were captured of each eye. A digital pulsimeter (TN1012/ST; AD Instruments Pty Ltd., Colorado Springs, CO, USA) was placed on the index finger of the participants, which was then used to record the signal from the cardiac pulse using LabChart Pro (version 8.1.11; AD Instruments Pty Ltd., Sydney, Australia). The data acquisition from the pulsimeter and PhS-OCT was synchronized by using a trigger signal provided by an external controlling unit (ML866, PowerLab 4/30; AD Instruments Pty Ltd., New South Wales, Australia).

**Data Processing and Measurements of Pulsatile TM Motion**

Each dataset contained 2000 cross-sectional OCT images of the scanned area. Minimization of bulk tissue motion artifacts and automatic alignment of adjacent B-scans were achieved using a proprietary algorithm. The velocity waveform of each pixel in the OCT image was generated as described in our previous study. In brief: (1) the phase shift of each pixel in the OCT signal was calculated between adjacent B-scans, and then the instantaneous velocity was obtained by calculating the displacement between two B-scan images; (2) the velocity waveform during the scan period (5 seconds) was acquired on every pixel in the PhS-OCT image; (3) motion waveforms of every location were estimated, please refer to the papers by Wang et al.
generated in whole OCT image followed by use of a mask derived from the cardiac pulse and harmonic frequency filtered the motion waveforms in the frequency domain. The maximum velocity (MV) was defined as the maximum value on the velocity waveform, and cumulative displacement (CDisp) was defined as the integration of the velocity waveform within a cardiac cycle.

The PhS-OCT images were analyzed to determine both MV and CDisp of the pulsatile TM motion as follows: (1) a line was drawn between the scleral spur and Schwalbe’s line on the interior inner surface of the cornea; (2) the internal inner region of the TM was chosen at one-third of the distance anterior to scleral spur along the line described in (1), then the value of internal MV (IMV) and internal CDisp (ICDisp) was calculated; (3) the external region of the TM was identified at the area next to Schlemm’s canal opposite the internal location, then the value of the external MV (EMV) and external CDisp (ECDisp) were calculated; and (4) the values for MV and CDisp were calculated as the average of three acquired consecutive PhS-OCT images. The mean value for MV and CDisp of each eye was calculated as the average of the internal and external region of the TM, which were defined as MVmean and CDispmean. All measurements were performed by an experienced investigator (K.G.), who was masked to the subject’s clinical data.

Image analysis to measure the MV and CDisp of the TM motion was performed in two separate sessions over an interval of 2 weeks to assess the reproducibility of the pulsatile TM motion parameter measurements.

Statistical Analysis

Descriptive statistics were calculated as the means and standard deviations (mean ± SDs). The significance of differences in parameters of pulsatile TM motion between normal and patients with POAG were determined using either an unpaired t-test or a Mann–Whitney U test based on the analysis of normality (Shapiro–Wilk test). The intraclass correlation coefficient (ICC) was used to demonstrate the repeatability and reproducibility of the pulsatile TM motion measurements in PhS-OCT images. Correlation analysis among pulsatile TM motion parameters (i.e. IMV, EMV, and MVmean, ICDisp, ECDisp, and CDispmean) and other ocular parameters (i.e. outflow facility and ocular pulse amplitude) was determined using Pearson correlation test. Receiver operating characteristic (ROC) curves were generated to compare the glaucoma detection sensitivity of various parameters. From the ROC curves, we obtained the area under the curve (AUC) of each of the parameters. Statistical analyses were performed using SPSS software version 25.0 (SPSS, Inc., Chicago, IL, USA). P < 0.05 indicated statistical significance. In correlation analysis, significant probability values obtained were analyzed for multiple testing using Bonferroni correction (P < 0.05/3 indicating statistical significance).

RESULTS

Demographic and Baseline Characteristics of the Study Subjects

This study consecutively enrolled eight healthy individuals and nine patients with POAG. The mean age of healthy and patients with POAG was 63.1 ± 4.0 years (range = 59–71 years) and 65.6 ± 10.2 years (range = 47–83 years), respectively. Mean ± SD IOPs of the healthy individuals and patients with POAG were 14.0 ± 2.7 mm Hg and 14.3 ± 3.3 mm Hg on an average of 1.6 ± 1.0 IOP-lowering medications, respectively. Baseline characteristics of all participants are shown in Table 1.

Repeatability and Reproducibility of the Measurements of Pulsatile TM Motion

ICCs were assessed to test the agreement of all measurements. The ICCs of IMV, ICDisp, EMV, and ECD for the three consecutive scans in the same eye were 0.965, 0.991, 0.952, and 0.96, respectively, which suggested good repeatability of our measurements. Moreover, the ICCs of IMV, ICDisp, EMV, and ECD, which were analyzed twice on 2 separate sessions over an interval of 2 weeks, were 0.98, 0.972, 0.979, and 0.97, respectively, which also represented a high-level of reproducibility. Because of the high repeatability and reproducibility of the measurements in both our present study and a previous study,12 we felt that it was satisfactory to use data obtained by one observer (K.G.).

Difference in MV and CDisp Between Healthy Eyes and Eyes with POAG

Figure 2 shows the difference in MV and CDisp between a representative healthy subject and an eye with POAG. Both the maximum velocity and motion amplitude of the TM in the eye with POAG was significantly lower than that in the healthy eye (Supplementary Videos S1 and S2). Box plots showed the measured MV and CDisp for each individual in both groups (Fig. 3). Table 2 summarizes the results for parameters of pulsatile TM motion in the internal and external region of all the recruited eyes in our study. In both regions of the TM, the MV and CDisp in healthy eyes were each significantly higher than those in eyes with POAG (all P < 0.05); the mean MVmean and CDispmean difference in the result was similar (22.4 ± 5.2 μm/s vs. 13.1 ± 2.6 μm/s; P < 0.001 for MVmean; and 0.414 ± 0.25 μm vs. 0.195 ± 0.05 μm; P < 0.001 for CDispmean). Although the motion signals
TABLE 1. Demographics and Clinical Characteristics

| Variable                     | Healthy Eyes | POAG Eyes     | P Value                  |
|------------------------------|--------------|---------------|--------------------------|
| Age, y                       | 63.1 ± 4.0   | 65.6 ± 10.2   | 0.53                     |
| Axial length, mm             | 23.5 ± 0.9   | 24.4 ± 1.2    | 0.025                    |
| Anterior chamber depth, mm   | 5.1 ± 0.3    | 3.3 ± 0.4     | 0.06                     |
| Cup-to-disc ratio            | 0.32 ± 0.1   | 0.82 ± 0.1    | <0.01                    |
| Mean arterial pressure, mm Hg| 98.0 ± 11.4  | 95.1 ± 17.1   | 0.69                     |
| Heart rate                   | 67.5 ± 11.2  | 63.9 ± 14.3   | 0.57                     |
| Intraocular pressure, mm Hg  | 14.0 ± 2.7   | 14.3 ± 3.3    | 0.75                     |
| Outflow facility, μL/min/mm Hg| 0.42 ± 0.3  | 0.46 ± 0.4    | 0.76                     |
| Ocular pulse amplitude, mm Hg| 3.85 ± 1.4   | 3.88 ± 1.5    | 0.96                     |

POAG, primary open-angle glaucoma; SD, standard deviation.
* Unpaired t-test.

![FIGURE 2. Pulsatile trabecular meshwork (TM) movement waveform revealed by phase-sensitive optical coherence tomography (PhS-OCT). Shown are the representative results of pulsatile TM motion in a healthy eye and an eye with POAG. In the healthy eye (A, B, C), instantaneous pulsatile motion of the TM in systole and diastole phases are shown in B and C, respectively. Waveform shown in black lines in A and (D) are the pulsimeter signal. The color heat map illustrates the direction and intensity of the tissue movement. The red color represents the movement anteriorly toward the surface of cornea, whereas the blue color represents the movement posteriorly toward the anterior chamber angle. (E, F) show the instantaneous pulsatile motion in an eye with POAG. A, D provide the results of pulsatile TM motion in a healthy eye and an eye with POAG averaged over 5 seconds, respectively. The maximum velocity (green arrow) and motion amplitude (orange line) of the TM in the eye with POAG was significantly lower than that in the healthy eye. POAG, primary open-angle glaucoma; AC, anterior chamber; maxVel, maximum velocity; # Disp, cumulative displacement; Disp, displacement.

of background tissue (i.e. corneoscleral coats) appear to be relatively random, the averaged displacements from both normal and POAG patients were approximately 0.1 μm, markedly smaller than the motion detected at the TM region (Supplementary Fig. S1).

Correlation Analysis Between Pulsatile TM Motion Parameters and Other Ocular Measurements

Pearson's correlation analysis was conducted to determine correlations between pulsatile TM motion parameters and other ocular measurements. Our study found no correlations between pulsatile TM motion parameters and outflow facility. Although MV did not show a good correlation with ocular pulse amplitude, we found a significantly positive correlation between the CDisp and ocular pulse amplitude in all subjects (Pearson r = 0.50; P = 0.0038 for ICDisp; Pearson r = 0.42; P = 0.0017 for ECDisp; Pearson r = 0.46; P = 0.0088 for CDispmean; and P < 0.0167 indicating statistical significance after Bonferroni correction).

Glaucma Detection Sensitivity of Various Monitoring Parameters

Figure 4 shows the area under the ROC curves using the values of outflow facility, MV, and CDisp to identify healthy subjects and patients with glaucoma. The ability to discriminate glaucoma from normal parameters was assessed by calculating the AUC; the higher value suggests better discrimination. The diagonal reference line represents a random distribution with an absence of any relationship of clinical value. The outflow facility showed a relatively low
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Figure 3. Statistical analysis showing measured maximum velocity (A) and cumulative displacement (B) for each individual in the healthy group and POAG group. The mean maximum velocity and cumulative displacement in healthy eyes were significantly higher than those in eyes with POAG. POAG, primary open-angle glaucoma; CDisp, cumulative displacement; MV, maximum velocity. ***P < 0.001.

Table 2. Comparison of MV and CDisp Between Normal Eyes and Eyes With POAG

| Variable                | Normal Eyes | POAG Eyes  | P Value |
|-------------------------|-------------|------------|---------|
| Internal region of the TM |             |            |         |
| IMV, μm/s               | 19.4 ± 4.7  | 10.9 ± 2.3 | <0.001  |
| ICDisp, μm              | 0.33 ± 0.19 | 0.16 ± 0.05| <0.001  |
| External region of the TM |           |            |         |
| EMV, μm/s               | 25.3 ± 5.8  | 15.3 ± 3.1 | <0.001  |
| ECDisp, μm              | 0.50 ± 0.33 | 0.23 ± 0.05| <0.001  |

MV, maximum velocity; CDisp, cumulative displacement; POAG, primary open-angle glaucoma; SD, standard deviation.

DISCUSSION

In this study, we found that eyes with POAG had significantly less pulsatile TM motion compared with healthy eyes using PhS-OCT. Previously, PhS-OCT was used to characterize the parameters of pulse-dependent TM motion and demonstrate its change with accommodation in healthy subjects. The newly observed difference between healthy eyes and eyes with POAG found in this study may be useful in understanding the functional behavior of the AH outflow pathways and in the evaluation and management of glaucoma in clinical practice.

We performed a correlation analysis between parameters of pulsatile TM motion and outflow facility as well as ocular pulse amplitude. CDisp showed good correlation with ocular pulse amplitude whereas MV and CDisp did not correlate with outflow facility, which may be due to some limitations of tonography in clinical practice. Significantly improved performance of MV and CDisp AUCs were observed compared with those of either outflow facility or IOP. Tonographic measurement limitations are well known and provide an explanation for the lack of routine use in clinical practice. The difference of glaucoma detection ability that we found suggests PhS-OCT may prove to be a more effective tool than tonography for the evaluation of functional properties of the outflow system.

The performance of the AUC for IOP was also less satisfactory than pulsatile parameters in this study, demonstrating its lack of an ability to predict the glaucoma status in contrast to the PhS-OCT measurements. The reason for the
lack of a difference in IOP between the healthy subjects and the patients with POAG may be the use of IOP-lowering medications in glaucoma eyes in our study. In addition, there is ample evidence to suggest that in-office random and infrequent IOP measurements are a poor surrogate for IOP levels throughout the 24 hour period and across multiple visits. Our results are consistent with the evidence that random IOP measurements may not be reflective of the real-time ability of the outflow system to maintain IOP within a narrow range. The pulsatile TM parameters had superior predictive capabilities compared with both outflow facility and the IOP in this study. We recognize that the limited number of subjects in our study permits only a preliminary conclusion, but we feel the findings point to the value of pursuing additional studies.

The ocular pulse results from cardiac cycle induced oscillatory changes in choroidal volume. The pulse waves are transmitted to the entire corneoscleral shell, which must undergo continuous cyclic distention and recoil. However, cyclic motion of the corneoscleral shell was too small for our system to detect. The ocular pulse amplitude reflects the strength of ocular blood flow corresponding to the difference between the minimum and maximum of the pulse wave contour. Similarly, the cumulative displacement is the integration of the minimum and maximum of the pulsatile motion corresponding to a cardiac cycle, which consists of both the diastolic and systolic phase. The differences between the analysis associated with velocity and displacement might be the reason CDisp, but not the MV, had a positive correlation with the ocular pulse amplitude. The MV only provided the maximum value of the instantaneous velocity on the velocity waveform.

Previous studies have shown structural differences in the TM between healthy eyes and eyes with POAG using scanning electron microscopy, atomic force microscopy, high-frequency ultrasound biomicroscopy, and OCT. Results of the present clinical research OCT study extend the knowledge to a new parameter; a functional property related to biomechanical changes in vivo in the TM of glaucomatous eyes. Pulse-dependent motion of the TM originates from the cardiac pulse through the changes of choroidal vascular volume between systolic and diastolic cardiac pulse waves. This pulsatile movement of trabecular meshwork and collector channel in healthy eyes was documented in a recent study using PhS-OCT. Our work is based on the concept of the aqueous pump model with the mechanism described in the latest paper by Lusthaus et al. In general, stiffness of the TM depends on the mechanical properties of the sensory arm of the TM cells and their ability to transduce sensory stimuli to alter their own properties as well as their ability to maintain optimal properties of the TM beams and other extracellular matrix components. According to the principles of biomechanics, a stiffer TM would exhibit less tissue deformation than the normal TM.

The pulsatile movement of the TM due to the pressure difference between the anterior chamber and Schlemm’s canal results in pulse-dependent distention and recoil. Therefore, an alteration in the composition of the beams, and an accumulation of ECM in the TM interspaces may compromise the pulsatile TM motion in eyes with POAG. Moreover, we speculate that the compromised pulsatile movement of the TM may induce changes in mechanosensing of shear stress or mechanical stretching of the TM thus establishing a positive feedback loop. Recent study has shown that SC shear stress and TM strain may act as mechanosensory factors for homeostatic regulation of outflow resistance and IOP. This feedback loop may exacerbate malfunction of the TM cells, cause defective modulation of ECM of the TM beams, and also result in excessive accumulation of ECM in the spaces between the beams and in the JCT. Together, these changes may lead to an increase in the resistance to AH outflow and elevation of IOP.

Depending on differences in outflow facility through the 360 degree circumference of the TM, segmental flow and nonuniform flow patterns were found in both the TM region and distal outflow system. Huang and colleagues demonstrated the segmental pulsatile outflow patterns using aqueous angiography in living nonhuman primates and in living human subjects. Recently, the TM-targeted microinvasive glaucoma surgery (MIGS) and new IOP-lowering drugs have received increasing attention from researchers. For instance, a trabecular micro-bypass system was implanted through the TM into Schlemm’s canal, resulting in improvement of AH outflow and a reduction of IOP. MIGS implants were shown to be safer and had a smaller surgical incision compared with conventional glaucoma surgeries in recent studies. Ideally, the PhS-OCT technique could examine 360 degrees of the limbal circumference preoperatively to determine desirable regions for MIGS usage. Distal outflow is more likely to be intact in regions exhibiting normal pulsatile motion, which may provide better outcomes. On the other hand, it might be better to treat an area without good pulsatile movement, thus sparing the area with more normal function. The optimal strategy for this awaits further study. Theoretically, with PhS-OCT, we could accurately select the TM region based on an optimized strategy, thus offering the potential for customizing TM-based glaucoma surgeries.

Moreover, Rho-associated protein kinase (ROCK) inhibitors were developed as a novel class of medications to lower IOP in patients with glaucoma, and were approved for the treatment of glaucoma in the United States in 2017. These ROCK inhibitors act to significantly increase outflow facility or decrease outflow resistance (i.e. decreasing the density of actin stress fibers, modifying the cytoskeleton of the TM cells to be less stiff, and relaxing smooth muscle cells in the TM region). Although IOP is an indirectly derived surrogate for the behavior of the outflow system, it does not provide a definitive functional assessment. Therefore, real-time TM-targeted parameters other than IOP are needed to evaluate the changes in the TM behavior following the use of novel IOP-lowering medications and MIGS. PhS-OCT, through its ability to detect dynamic TM motion represents such a parameter. Our future studies will focus on evaluation of the pulsatile motion over the entire circumferential TM tissue and changes in TM pulsatile movement in patients with glaucoma as a result of instillation of TM-targeted glaucoma medications and MIGS.

Limitations

There are several limitations in this study. (1) Our present study investigated the difference in pulsatile TM motion between healthy eyes and eyes with POAG. Whether there are changes in the pulsatile movement of the TM in patients with glaucoma and in different stages of POAG will require further cross-sectional and longitudinal studies. (2) Another potential limitation of our study was that the patients with glaucoma were all under treatment with pressure-lowering medications. The medications may affect not only pressures.
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and facility but also the biomechanics related to TM motion. Despite the presence of medication-lowering agents and lower pressures, PhS-OCT was effective in identifying patients with glaucoma. The study suggests that neither lower IOP nor the presence of medications restores abnormal biomechanical properties identified by our studies of TM motion. An additional limitation of this initial feasibility study is that we only examined the temporal quadrant. The results of this study appear sufficiently promising that we are planning a future study to determine variability of motion around the entire circumference of the limbus. Last, repeatability in this study was assessed using a single operator; future studies will also be needed to assess inter-user repeatability.

CONCLUSIONS

In this study, pulsatile TM motion in healthy eyes was found to be significantly greater than in glaucoma eyes using PhS-OCT. The reduced motion may be due to the altered tissue stiffness or other biomaterial properties of the TM in eyes with POAG. Imaging the pulsatile TM motion may provide new insights into glaucoma pathophysiology and may also be useful in the clinical management of glaucoma.

Acknowledgments

The authors thank the coordination assistance of Francy Moses for this study.

Meeting presentation: Presented in part at the Association for Research in Vision and Ophthalmology Annual Meeting, Vancouver, BC, Canada, April 28 to May 2, 2019.

Supported by the Washington Research Foundation (Wang), Carl Zeiss Meditec Inc. (Wang), Latham grant (Joanne C. Wen), the Fundamental Research Funds of the State Key Laboratory of Ophthalmology (30300602040020315), the Medical Scientific Research Foundation of Guangdong Province (A2020075), and an unrestricted departmental grant from Research to Prevent Blindness.

Role of the Funder/Sponsor: The funder had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

Disclosure: K. Gao, None; S. Song, None; M.A. Johnstone, None; Q. Zhang, None; J. Xu, None; X. Zhang, None; R.K. Wang, Oregon Health and Science University and the University of Washington (N), Carl Zeiss Meditec Inc. (F), Moptim Inc. (F), Colgate Palmolive Company (F), and Facebook Technologies LLC (F), Carl Zeiss Meditec (C), Johnson & Johnson Vision Care (C), and Insight Photonic Solutions (C). J.C. Wen, None

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**SUPPLEMENTARY MATERIAL**

**SUPPLEMENTARY VIDEOS S1 AND S2.** The supplementary videos show dynamic tissue displacements experienced by the TM relative to motion of the surrounding tissue over a period of 3 seconds. The TM clearly experiences pulsatile motion identical to the heart-beat frequency. The patient with POAG shows a lower magnitude of motion compared to the healthy subject.