Peripapillary Microvascular Improvement and Lamina Cribrosa Depth Reduction After Trabeculectomy in Primary Open-Angle Glaucoma

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PURPOSE. To evaluate peripapillary microvascular changes in patients with primary open-angle glaucoma (POAG) after trabeculectomy using optical coherence tomography (OCT) angiography, and to determine the influence of lamina cribrosa (LC) displacement on changes in peripapillary microvasculature.

METHODS. The peripapillary retinal microvasculature and LC were imaged using OCT angiography and OCT-enhanced depth imaging, respectively. The microvasculature and LC depth (LCD) were measured before, and 1 week, 1 month, and 3 months after trabeculectomy. The microvascular improvement was arbitrarily defined as a reduction >30% of the area of vascular dropout (blue/black areas with <20% vessel density on the color-coded vessel density map). LCD was determined as the mean of vertical distance between the anterior LC surface and a reference plane of Bruch’s membrane.

RESULTS. Thirty-one eyes of 31 POAG patients were included. At 3 months postoperatively, intraocular pressure (IOP) and LCD were significantly decreased from 26.3 ± 11.8 mm Hg to 12.5 ± 3.6 mm Hg, and 501.1 ± 130.2 μm to 455.8 ± 112.7 μm, respectively (all P < 0.001), compared with baseline. The microvascular improvement was observed in 19 eyes (61.3%) at 3 months after trabeculectomy. The maximal reductions in IOP and LCD were significantly greater in eyes with improved microvasculature compared to eyes without improvement (P = 0.020 and P = 0.005). The microvascular improvement was significantly associated with maximal reduction in LCD (odds ratio, 1.062; P = 0.026).

CONCLUSIONS. Trabeculectomy can improve peripapillary retinal microcirculation in patients with POAG. This finding suggests that the reduction of LCD induced by lowering IOP may affect peripapillary microvascular improvement in eyes with POAG.

Keywords: glaucoma, trabeculectomy, intraocular pressure, optical coherence tomography angiography, lamina cribrosa

Elevated intraocular pressure (IOP) is an important risk factor for the development and progression of glaucoma.1–4 The mainstay of glaucoma treatment is lowering of IOP to prevent disease progression.5 Trabeculectomy can be considered if IOP is not adequately controlled or glaucomatous damage progresses despite maximally tolerated medical therapy (MTMT). Recent studies have reported that position of the lamina cribrosa (LC) can be changed after surgical lowering of IOP, and that the reversal of LC displacement is related to a slower rate of disease progression.6,7 The effect of IOP-related stress on the LC may induce occlusion of the laminar capillaries and axonal ischemia; therefore, it is believed that the change of LC position would provide relief to compressed capillaries in the LC.8

Ocular hemodynamic alteration after surgical IOP lowering has been demonstrated in previous studies,9–13 most of which reported an increase in ocular blood flow parameters in patients with glaucoma after a significant drop in IOP after trabeculectomy. The decrease in IOP with subsequent increase in ocular perfusion pressure may enhance ocular blood flow after trabeculectomy.9 The retinal microcirculation is also expected to be improved by trabeculectomy; however, this has yet to be confirmed.

Recently, it has become possible to visualize microvascular networks of the retinal capillaries using optical coherence tomography angiography (OCTA). This technique provides reproducible and quantitative information about the peripapillary retinal microvasculature.14 Recent OCTA studies revealed that microvascular impairment in glaucomatous eyes was associated with LC deformation.15,16 Hence, we sought to test the hypothesis that retinal microcirculation would be improved by surgical lowering of IOP. We evaluated peripapillary microvascular changes in patients with primary open-angle glaucoma (POAG) after trabeculectomy using OCTA. We also investigated the influence of LC position on peripapillary microvascular changes.

METHODS

This prospective, observational study consecutively recruited patients with POAG who were scheduled for trabeculectomy at
the glaucoma clinic of Asian Medical Center (Seoul, Korea), between October 2016 and April 2017. The Institutional Review Board of Asian Medical Center approved this study, and the study design was executed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all individuals who underwent trabeculectomy before participation.

At the initial evaluation, all subjects underwent a comprehensive ophthalmologic examination, including a review of medical history; best-corrected visual acuity assessment; refraction test; slit-lamp biomicroscopy; Goldmann applana-
tion tonometry; gonioscopy; central corneal thickness assessment (DGH-550; DGH Technology, Inc., Exton, PA, USA); axial length measurement (IOLMaster; Carl Zeiss Meditec, Dublin, CA, USA); fundoscopic examination; stereoscopic optic disc photography; red-free photography; standard automated perimetry (Humphrey Field analyzer with Swedish Interactive Threshold Algorithm standard 24-2 test; Carl Zeiss Meditec); spectral-domain (SD)-OCT (Avanti RTVue-XR; Optovue, Inc., Fremont, CA, USA); and OCT-A (AngioVue; Optovue Inc.).

To be included, all subjects were required to have POAG, a best-corrected visual acuity of 20/40 or better, a spherical refraction of –8.0 to +3.0 diopters (D), a cylinder correction within ±3 D, and clear ocular media. POAG was defined as having an open angle on gonioscopy, history of IOP >21 mm Hg, retinal nerve fiber layer defects or glaucomatous optic disc changes (neuroretinal rim thinning, disc excavation, or disc hemorrhage), and corresponding visual field (VF) defects confirmed by at least two reliable VF examinations. Only reliable VF test results (i.e., false-positive errors <15%, false-negative errors <15%, and fixation loss <20%) were included in the study. A glaucomatous VF defect was defined as: the presence of a cluster of ≥5 nonedge contiguous points on a pattern deviation plot with $P < 5\%$ (1 of which had a $P < 1\%$) confirmed by at least two consecutive examinations; a pattern standard deviation with $P < 5\%$; or a glaucoma hemifield test result outside normal limits. Patients with any ophthalmic or neurologic disease known to affect the optic nerve head or VF were excluded.

The indications for trabeculectomy were based on the progression of glaucomatous damage (VF and/or optic disc) and/or elevated IOP despite MTMT. All ocular hypotensive medications were continued up to the time of surgery. Patients with hypotony maculopathy or extremely low IOP of less than 6 mm Hg after trabeculectomy were excluded from this study. If both eyes met the inclusion criteria, one eye was randomly chosen for analysis. The IOP measurement, OCT-enhanced depth imaging, and OCT-A imaging were performed at 1 week, and 1 and 3 months after surgery.

**OCT-A Imaging**

The OCTA imaging system (AngioVue; Optovue, Inc.) provides noninvasive visualization of the retinal microvasculature. A split-spectrum amplitude-decorrelation angiography algorithm was used to identify perfused vessels by capturing the dynamic motion of moving particles, such as red blood cells. Details of this algorithm have been previously described.\(^1\)\(^\text{1}^\) In this study, each patient underwent peripapillary OCT-A imaging of a 4.5 × 4.5 mm region centered on the optic disc. The microvascular information is presented as a vessel density map or color-coded vessel density map in the whole retinal layer between internal limiting membrane and retinal pigment epithelium. It also provides a quantitative vessel density (%) measurement, calculated as the percentage of measured area occupied by vessels with flowing blood. Circumpapillary vessel density (cpVD) was measured in a region defined as a 750 μm-wide elliptical annulus extending from the optic disc boundary.

All scans were individually reviewed by two investigators (JWS, KRS) for evaluation of quality. Eyes with poor image qualities were excluded on the basis of the following criteria\(^18\),\(^19\): signal strength index (SSI) <40; poor-clarity images; localized weak signal caused by artifacts such as floaters; residual motion artifacts visible as irregular vessel patterns or disc boundary on the enface angiogram; or segmentation failure.

**Determination of Microvascular Improvement**

To determine microvascular improvement, the vascular dropout (red regions) was compared between the color-coded vessel density maps before and 3 months after trabeculectomy. The vascular dropout was defined as blue/black areas with <20% vessel density on the color-coded vessel density map. The microvascular improvement was defined as a reduction of >30% of the area of vascular dropout. The postoperative image was registered to the preoperative image, then same regions (green regions) were compared. In this case, the area of vascular dropout was decreased from 6.75 mm\(^2\) to 4.44 mm\(^2\) (34.2% reduction) after trabeculectomy and, therefore, is considered to be an improvement.

![Before surgery](image1.png)

![Three months after surgery](image2.png)

**FIGURE 1.** To determine microvascular improvement, the vascular dropout (red regions) was compared between the color-coded vessel density maps before and 3 months after trabeculectomy. The vascular dropout was defined as blue/black areas with <20% vessel density on the color-coded vessel density map. The microvascular improvement was defined as a reduction of >30% of the area of vascular dropout. The postoperative image was registered to the preoperative image, then same regions (green regions) were compared. In this case, the area of vascular dropout was decreased from 6.75 mm\(^2\) to 4.44 mm\(^2\) (34.2% reduction) after trabeculectomy and, therefore, is considered to be an improvement.

To determine microvascular improvement, the vascular dropout was compared between the color-coded vessel density maps before and 3 months after trabeculectomy. In a previous OCTA study, regional vessel density in patients with moderate-to-advanced glaucoma ranged 26.1% to 40.9%.\(^2^0\) Based on this study, the vascular dropout was arbitrarily defined as blue/black areas with <20% vessel density on the color-coded vessel density map (Fig. 1). The area of vascular dropout was measured by a computer program written using commercial software (MATLAB; The MathWorks, Inc., Natick, MA, USA). To determine the tolerable threshold of test-retest variability, OCT-A imaging was repeated twice with 1-month interval in 22 POAG eyes who had continued topical IOP-lowering treatment without surgical intervention. The area of vascular dropout was 6.3 mm\(^2\) at baseline test and 6.0 mm\(^2\) at 1-month follow-up test. The difference of vascular dropout area between test-retests was $-0.3 \pm 0.6$ mm\(^2\). We calculated the tolerable threshold using the formula $1.645 \times \sqrt{2 \times \text{test-retest standard deviation}}$\(^21\). The tolerable threshold of vascular dropout area was 1.39 mm\(^2\) (22.1%). Hence, microvascular improvement in OCTA was arbitrarily defined as a reduction >30% of the area of vascular dropout to avoid test variability being labelled as improvement. In order to compare same area, the color-coded
vessel density map at 3 months after trabeculectomy was registered to the preoperative image using MATLAB.

**Measurement of LC Depth and Choroidal Thickness**

The LC was imaged at 4.5 × 4.5 mm region (304 × 304 A-scans) using the enhanced depth imaging technique with SD-OCT (Avanti RTVue-XR; Optovue, Inc.). Details of this technique to evaluate the LC have been described previously,22,23 To determine LC depth (LCD) and choroidal thickness, 7 B-scans were selected from the three-dimensional image dataset of SD-OCT. Seven B-scan images spaced equidistantly across the vertical optic disc diameter. The anterior LC surface was delineated manually in all B-scans. The Bruch’s membrane positions were marked at 500 μm from the termination of the Bruch’s membrane and used to create reference plane. The LC was defined as the vertical distance between the anterior LC surface and reference plane. Only the temporal part of the LC from the maximally depressed point was used for LCD measurement. In each B-scan, the average LCD was calculated by the mean of depth measurements with reference plane. The choroidal thickness (white arrows) was measured at every 500 μm from the end of the Bruch’s membrane in each B-scan. Measurements from seven B-scans were averaged and defined as the LCD and choroidal thickness of the eye.

**Statistical Analysis**

Normality of the data was confirmed in all continuous variables by the Kolmogorov-Smirnov test. The clinical characteristics were compared between eyes with improved and non-improved peripapillar microvasculature after trabeculectomy using the independent t-test for continuous variables and χ² test for categorical variables. The preoperative and postoperative IOP, LCD, and cpVD values were compared using repeated measures analysis of variance (rANOVA) in the improved and non-improved groups, respectively. Univariate logistic regression analysis was used to determine the factors associated with the improved peripapillary microvasculature after trabeculectomy. Then, stepwise multivariate logistic regression was performed for variables with a P value < 0.10 in the univariate analysis. Statistical analysis was performed using statistical software (SPSS version 20; IBM Corp., Armonk, NY, USA). P value ≤ 0.05 was considered to be statistically significant.

**RESULTS**

A total of 45 eyes of 45 POAG patients who underwent trabeculectomy were initially enrolled. Of these, 11 subjects were excluded due to poor image quality and 3 were excluded due to extremely low postoperative IOP. A total of 31 eyes of 31 POAG patients (20 male, 11 female) who underwent trabeculectomy were included in the final analysis. At the baseline examination, the mean age, refractive error, axial length, central corneal thickness, IOP, and VF MD were 56.1 ± 12.1 years, −1.46 ± 2.53 D, 24.71 ± 1.65 mm, 549.2 ± 30.2 μm, 26.3 ± 11.8 mm Hg, and −17.25 ± 11.79 dB, respectively. The interobserver ICCs for measurement of the LCD and choroidal thickness was 0.942 (95% confidence interval [CI] 0.898–0.981; P < 0.001) and 0.960 (95% CI 0.931–0.985; P < 0.001), respectively. At 3 months postoperatively, the IOP and LCD were significantly decreased from 26.3 ± 11.8 mm Hg to 12.5 ± 3.6 mm Hg and 501.1 ± 130.2 μm to 455.8 ± 112.7 μm, respectively (all P < 0.001, rANOVA). Although the cpVD and choroidal thickness were increased from 44.9% ± 6.0% to 47.0% ± 7.2%, and 130.9 ± 44.4 μm to 138.4 ± 51.9 μm, respectively, this difference was not statistically significant (P = 0.133 and 0.127, respectively).

**FIGURE 2.** To determine LCD, seven B-scans (green line) were selected from the three-dimensional image dataset of SD-OCT. Seven B-scan images were spaced equidistantly across the vertical optic disc diameter. The Bruch’s membrane position was marked at 500 μm from the termination of Bruch’s membrane and used to create reference plane (red dotted lines). The LCD was defined as the vertical distance between the anterior LC surface and reference plane. Only the temporal part of the LC from the maximally depressed point was used for LCD measurement. The choroidal thickness (white arrows) was measured at every 500 μm from the end of the Bruch’s membrane in each B-scan. Measurements from seven B-scans were averaged and defined as the LCD and choroidal thickness of the eye.
The peripapillary microvascular improvement was observed in 19 (61.3%) eyes at 3 months after trabeculectomy. Table 1 summarizes the clinical characteristics of eyes with and without microvascular improvement. There were no significant differences in IOP, LCD, choroidal thickness, cpVD, and SSI between eyes with and without microvascular improvement during entire follow-up period. However, maximal reductions in IOP and LCD were significantly greater in eyes with microvascular improvement than eyes without (P = 0.020 and P = 0.005, respectively).

Figure 3 describes the measurements of IOP, LCD, and cpVD at each follow-up visit according to microvascular changes. The IOP was significantly decreased through entire postoperative period compared to preoperative IOP, regardless of microvascular improvement (all P < 0.05). Similarly, a significant reduction in LCD was observed during entire postoperative period, regardless of microvascular improvement (all P < 0.05). The cpVD exhibited a significant increase at 1 and 3 months postoperatively in eyes with microvascular improvement (P = 0.008 and 0.015, respectively).

The results from the logistic regression analysis examining peripapillary microvascular improvement after trabeculectomy are summarized in Table 2. Based on the univariate logistic analysis, microvascular improvement was significantly associated with maximal reductions in IOP (odds ratio [OR], 1.154; P = 0.025) and LCD (OR, 1.049; P = 0.019). Image quality (i.e., SSI) showed borderline significance (OR, 0.941; P = 0.074). The multivariate logistic regression was performed for variables with a P value <0.10 in the univariate analysis. In multivariate analysis, microvascular improvement was significantly associated with maximal reduction in LCD (OR, 1.062; P = 0.026).

**Representative Case**

Figure 4 depicts a representative case involving a 54-year-old woman with POAG (preoperative IOP, 32 mm Hg; VF MD, −21.11 dB) who demonstrated microvascular improvement after trabeculectomy. The postoperative IOP was decreased to 9 mm Hg at 1 week, then gradually increased to 11 mm Hg at 1 month, and 14 mm Hg at 3 months. During the 3-month follow-up period, the retinal microvasculature was gradually improved at the area of vascular dropout with a significant reduction in LCD.

**DISCUSSION**

In the current study, we observed peripapillary microvascular improvement in 61.3% of eyes after trabeculectomy. More importantly, the most relevant factor for microvascular improvement after surgery was the amount of maximal reduction in LCD. The posterior displacement of the LC is...
TABLE 2. Factors Associated With Peripapillary Microvascular Improvement After Trabeculectomy

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<tr>
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<th>Multivariate</th>
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<tr>
<td></td>
<td>Odds Ratio (95% CI)</td>
<td>P</td>
<td>Odds Ratio (95% CI)</td>
<td>P</td>
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<tr>
<td>Age</td>
<td>1.003 (0.949–1.059)</td>
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<td>Axial length</td>
<td>0.804 (0.501–1.312)</td>
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<td>Central corneal thickness</td>
<td>0.989 (0.962–1.041)</td>
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<td>Visual field mean deviation</td>
<td>0.974 (0.894–1.062)</td>
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<td>Intraocular pressure</td>
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<tr>
<td>Baseline</td>
<td>1.034 (0.966–1.108)</td>
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<tr>
<td>Postoperative 1 week</td>
<td>1.092 (0.906–1.316)</td>
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<td>Postoperative 1 month</td>
<td>0.962 (0.743–1.246)</td>
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<td>Postoperative 3 month</td>
<td>1.024 (0.917–1.142)</td>
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<td>Maximum change</td>
<td>1.154 (1.008–1.136)</td>
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<td>1.116 (0.980–1.272)</td>
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<td>Lamina cribrosa depth</td>
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<tr>
<td>Baseline</td>
<td>0.982 (0.937–1.028)</td>
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<td>Postoperative 1 week</td>
<td>0.981 (0.934–1.030)</td>
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<td>Postoperative 1 month</td>
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<td>Postoperative 3 month</td>
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<td>Maximum change</td>
<td>1.049 (1.008–1.092)</td>
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<td>Choroidal thickness, μm</td>
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<tr>
<td>Baseline</td>
<td>0.995 (0.978–1.012)</td>
<td>0.549</td>
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<td>Postoperative 1 week</td>
<td>0.996 (0.981–1.011)</td>
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<td>0.995 (0.979–1.008)</td>
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<td>Maximum change</td>
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<td>Signal strength index</td>
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<td>Baseline</td>
<td>0.941 (0.875–1.013)</td>
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<td>1.014 (0.920–1.116)</td>
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<td>Postoperative 1 week</td>
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<tr>
<td>Postoperative 1 month</td>
<td>1.075 (0.966–1.198)</td>
<td>0.186</td>
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<td>Postoperative 3 month</td>
<td>1.097 (0.950–1.266)</td>
<td>0.209</td>
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</table>

FIGURE 4. A representative case involving a 54-year-old woman with POAG exhibiting gradual microvascular improvement (red arrows) after trabeculectomy. The retinal microvasculature was gradually improved at inferotemporal (postoperative 1 week); superonasal (1 month); and superior region (3 months) with reversal of LC. To determine the reversal of LC, the postoperative LCDs (red regions) were compared with the preoperative LCD (green region).
considered to be one of the important primary pathologic events in the development of glaucomatous optic nerve damage. LC deformation may suppress blood flow in the optic nerve bundle, resulting in ischemic insult to axons. Previous studies have comprehensively reported on the relief of mechanical stress by LC reversal after IOP-lowering treatment. Our findings demonstrated that reduction of LC depth after trabeculectomy may relieve microvascular suppression in the peripapillary region.

It is known that IOP reduction after trabeculectomy induces an increase in ocular perfusion pressure, thereby enhancing ocular blood flow. In the current study, we found significantly greater maximal IOP and LCD reduction in eyes with microvascular improvement after trabeculectomy than eyes without. However, there was no significant direct association between IOP reduction and the improvement in microvascular improvement in multivariate analysis, although both maximal changes in IOP and LCD demonstrated a possible association with microvascular improvement in univariate analysis. Moreover, no change or even temporary deterioration of microvasculature occurred in 38.7% of eyes after successful IOP reduction after trabeculectomy. Recently, Zeboulon et al. reported limited effect of surgically induced IOP reduction on peripapillary vessel density in OCTA. They observed the absence of modification of vessel density after surgical IOP reduction, although some cases (6 patients, 28.5%) showed an improvement in vessel density. This may indicate that IOP reduction itself does not work as a crucial factor in improving retinal microvasculature after surgical intervention. The individual susceptibility to IOP-related stress/strain is affected by various biomechanical factors involving the optic nerve head. IOP reduction by medical or surgical treatments does not necessarily induce LC reversal. Lee et al. reported that LC reversal was not observed in 41% of eyes after IOP-lowering treatment. Our findings may indicate that the microvascular improvement after trabeculectomy is better represented by changes in LCD rather than IOP itself.

Previous studies have reported that trabeculectomy improves retrobulbar blood flow according to color Doppler imaging, pulsatile ocular blood flow by fundus pulsation, and optic nerve head blood flow by scanning laser Doppler flowmetry. Recently, Hollo reported a case series (6 eyes of 4 subjects) with an increase in peripapillary capillary perfusion using OCTA after large medical IOP reduction. In addition to previous studies, we found improvement in retinal microvasculature after trabeculectomy. The retinal microvasculature, such as radial peripapillary capillaries, is the crucial source of nutrition for retinal ganglion cells and their axons. Hence, assessment of microvascular perfusion can be potentially useful for identifying and monitoring patients with glaucoma. Additionally, our findings suggest that OCTA is an effective approach to evaluate the postoperative microvascular status of the eyes that underwent trabeculectomy.

Trabeculectomy is effective in slowing the progression of VF damage in glaucoma patients. However, some cases continue to progress despite adequate surgical IOP control, suggesting that other factors contribute to progression of VF damage. Lee et al. reported that eyes with sustained, long-term LCD reduction exhibited a slow rate of progressive retinal nerve fiber layer thinning after trabeculectomy. Given that there was a close relationship between postoperative maximal reduction in LCD and microvascular improvement in the current study, vascular factors may play a role in the progression of glaucomatous damage after trabeculectomy. It would be important to investigate the progression of glaucomatous damage according to microvascular improvement after trabeculectomy.

This study had several limitations. The small number of patients may limit the generalizability of our findings to the entire population of patients with glaucoma. Our patients were observed for only up to 3 months after trabeculectomy. The IOP-lowering effect decreases gradually after surgery. A long-term sustained IOP reduction has been reported to be associated with a slow rate of progressive RNFL thinning after trabeculectomy rather than early postoperative LCD reduction. Therefore, further research is needed to determine whether microvascular improvement persists over a long-term follow-up period. Recently, it has been reported that LC curvature was reduced after trabeculectomy, and thus it would be interesting to explore the effect of LC curvature change on microvascular improvement in forthcoming study.

In conclusion, we demonstrated microvascular improvement in POAG patients 3 months after trabeculectomy using OCTA. The maximal reduction in LCD after surgery was significantly associated with microvascular improvement. The recovery of retinal microvasculature using surgical treatment may be supportive evidence of protection from the progression of glaucomatous damage. Further study investigating whether this microvascular improvement persists and whether it provides predictive insights for prognosis of glaucoma patients who undergo filtering surgery should be pursued.

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