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Quantitative and Morphological Corneal Endothelial Changes After Selective Laser Trabeculoplasty and Retinal Photocoagulation

*Aran Kanagaratnam, BSc**† *and Keith Ong, FRANZCO**†

Purpose: Selective laser trabeculoplasty (SLT) and retinal photocoagulation (RP) are two common laser procedures often performed at a wavelength of 532 nm, and may affect the corneal endothelium. This study used corneal specular microscopy to determine the impact of these procedures on the corneal endothelium.

Design: Retrospective cohort study in a private practice.

Methods: There were 249 eyes from 136 consecutive patients who underwent SLT for open-angle glaucoma and 132 eyes from 74 patients who underwent RP included. Corneal specular microscopy was performed immediately before and after each procedure and at 1-month postprocedure. Microscopy data included quantitative measures, such as cell density and central corneal thickness, and morphological measures, including percentage of hexagonal cells and coefficient of variation in cell area.

Results: There was a small (just over 1%) reduction in corneal endothelial cell count from pre-SLT to post-SLT ($P = 0.008$), and a statistically significant recovery at 1 month ($P = 0.04$). Central corneal thickness also transiently increased from pre-SLT to post-SLT ($P = 0.03$). Although polymegathism was unchanged, changes in pleomorphism were observed ($P = 0.03$). The only change in the RP group was an increase in polymegathism between pre-RP and post-RP ($P = 0.001$).

Conclusions: SLT has measurable effects on both quantitative and morphological characteristics of the corneal endothelium, which seem to be transient. RP has fewer measurable effects, likely because, although the total laser energy is similar, it is delivered over a much longer time (3 ns versus 0.1 s). The changes observed in both procedures are minor and unlikely to be of clinical significance.

Key Words: corneal endothelium, glaucoma, laser surgery

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Selective laser trabeculoplasty (SLT) and retinal photocoagulation (RP) are two common laser procedures frequently performed using a 532 nm (green light) laser.

SLT is a commonly performed therapeutic procedure for the treatment of open-angle glaucoma. It can be used either as a primary therapy or as an adjunctive therapy. Further, it has the advantage of not being reliant on patient compliance with eye drops, which has been reported to be low.[1] Compared with its predecessor, argon laser trabeculoplasty, SLT results in a lack of visible trabecular meshwork scarring and fewer side effects, while still resulting in a decrease in intraocular pressure comparable to argon laser trabeculoplasty in both newly diagnosed patients and patients with maximally tolerated medication.[2] Adverse effects as a result of SLT, such as corneal edema, are relatively uncommon and usually transient.[3]

RP is used in retinal ischemia, such as diabetic retinopathy and retinal vein occlusion, where the conversion of laser energy to thermal energy destroys ischemic peripheral retina, reducing the metabolic demands of the tissue and vascular endothelial growth factor production, and thus decreasing neovascularization.[4,5] RP can also be used to treat retinal holes and tears where slight inflammation and scarring cause choroid adhesion to the retina.[6] Adverse effects from RP include declines in visual acuity and visual field.[7]

In both SLT and RP, the corneal endothelium may be affected. In SLT, to reach the trabecular meshwork, laser energy must traverse the cornea, and in RP the laser energy passes through the cornea to reach the retina. The corneal endothelium consists of a single layer of nonregenerating, hexagonal cells that function to maintain corneal transparency by regulating the hydration of the cornea.[8] When the corneal endothelium is damaged, the normally small and regular hexagonal cells enlarge to cover the inner surface of the cornea and compensate for the loss of cells.[9] Corneal endothelial cell density decreases with age, and also after trauma to the cornea.[9]

Previous research has found the presence of transient "dark spots on corneal specular microscopy" that appear immediately post-SLT and seem to resolve by 1 month.[10,11] Furthermore, altered immunostaining for zonula occludens-1 (ZO-1) suggests that after SLT, corneal endothelial tight junctions may be compromised.[12] There have been mixed results regarding quantitative changes to corneal endothelial cell parameters, with Ong et al finding no difference to corneal cell density but a larger study by Lee et al finding a transient cell decrease at 1 week that seemed to recover by 1 month.[13,14]

Patients with diabetic retinopathy are known to have abnormally high levels of pleomorphism and polymegathism in the corneal endothelium.[15] Argon laser retinal photocoagulation (with a wavelength 514 nm) has been found to decrease endothelial density in patients with diabetic retinopathy.[16] However, it is not known whether modern RP, performed with a laser with a wavelength of 532 nm, will affect the corneal endothelium. Thus, the present study aimed to determine if there were demonstrable quantitative and morphological changes to the corneal endothelium from SLT or RP on specular microscopy.

From the *Department of Ophthalmology, Sydney Medical School, University of Sydney, Sydney, NSW, Australia; and †Department of Ophthalmology, Royal North Shore Hospital, Sydney, NSW, Australia
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Correspondence: Dr Keith Ong, 2 Railway Avenue, Eastwood, NSW 2122, Australia. E-mail: keithong@optusnet.com.au.

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TABLE 1. Patient Characteristics

Characteristic	SLT	RP
Demographics		
Participants	133	74
Total eyes undergoing SLT	244	132
Male participants (%)	61 (45.8)	36 (48.6)
Mean age, y	70.4 ± 11.4	64.7 ± 14.2
Mean number of glaucoma eye drops used	1.4 ± 0.9	NA
Ethnicity		
Asian (%)	105 (78.9)	61 (82.4)
Treatment eye		
Left eye (%)	123 (50.4)	64 (48.5)
Right eye (%)	121 (49.6)	68 (51.5)
Mean central corneal thickness	536 ± 38 μm	541 ± 34 μm
Treatment parameters		
Mean laser shots	61.1 ± 13.3 shots	NR
Mean peak laser energy	0.87 ± 0.24 mJ	NR
Mean total laser energy	40.6 ± 17.4 mJ	NR

NA indicates not applicable; NR, not recorded; RP, retinal photocoagulation; SLT, selective laser trabeculoplasty.

METHODS

This was a retrospective cohort study of patients and all procedures were performed by the same ophthalmologist (K.O.). From April 2017 to June 2018, 249 eyes from 136 consecutive patients who underwent SLT for open-angle glaucoma were included and 132 eyes from 74 patients who underwent RP for retinal conditions, including retinal holes, tears, or degeneration and diabetic retinopathy. Exclusion criteria included patients with concurrent corneal disease, such as Fuchs' corneal dystrophy. Patients taking medications affecting the cornea, such as amiodarone and hydroxychloroquine, were excluded. For the SLT cohort, patients were included who had open-angle glaucoma and where SLT was offered as an adjuvant intraocular pressure-lowering therapy. The protocol (Reference Code: RESP/18/154) was approved by the Northern Sydney Local Health District Human Research Ethics Committee and the tenets of the Declaration of Helsinki were adhered to (Tables 1–3).

The Ellex Tango (Ellex Medical Pty Ltd, Adelaide, Australia) laser was used for the SLT procedure. Power settings were titrated to 0.3–0.9 mJ to achieve the reaction of a hint of microbubbles in 80% of laser shots. About 60 ± 10 laser shots to 180 degrees of meshwork were done. Total laser energy, peak laser energy, and number of laser shots were recorded. Post-SLT all treated eyes were given a single drop of Maxidex, containing dexamethasone 0.1% (Alcon Inc, Fort Worth, Texas, USA), one drop of Acular, containing ketorolac tromethamine 0.5% (Allergan Inc, Irvine, California, USA), and one drop of Combigan containing combined timolol maleate 0.5% and brimonidine tartrate 0.2% (Allergan Inc, Irvine, California, USA). If patients were asthmatic, instead of Combigan, they were given one drop of Alphagan containing only brimonidine tartrate 0.2% (Allergan Inc, Irvine, California, USA).

The Ellex Solitaire (Ellex Medical Pty Ltd, Adelaide, Australia) laser was used for the RP procedure. The spot size was 300 μm and pulse duration 0.1 s, with power titrated to 200–400 mW and 150–500 shots given to achieve a light to medium white-grey burn.

Corneal specular microscopy was performed using the Tomey EM-3000 Corneal Specular Microscope (Tomey Corporation, Nagoya, Japan). The microscope was set to examine the central cornea, identified using the patient's fixation. Specular microscopy was performed immediately before and after each procedure (approximately 20 minutes postprocedure), and at 1-month post-procedure. Up to three images were taken, and image analysis was automated using the inbuilt software. All images were taken by the same ophthalmologist (K.O.). Images were not included for analysis if they included less than 75 cells in the image. This cut point was determined by balancing the fact that there is increased reliability from an image including more cells (ie, higher image quality) with the present study's cohort having a high average age and thus lower corneal endothelial cell density.[9,17]

Specular microscopy analysis was carried out as established by McCarey et al.[9] Parameters examined included corneal endothelial cell count, coefficient of variance of cell area, percentage of hexagonal cells, and central corneal thickness (CCT). All parameters are collated automatically by the microscope's inbuilt

TABLE 2. Corneal Cell Parameters Before and After SLT

	Pre-SLT	Post-SLT	1 Month	Significance
Endothelial cell density (cells/mm^2)	2292 ± 449	2265 ± 445	2279 ± 448	$P = 0.008$ for pre-SLT to post-SLT, $P = 0.04$ for post-SLT to 1 month. $P = 0.04$ for pre-SLT to 1 month
Coefficient of variance of cell area	38.5 ± 6.0	38.2 ± 6.9	38.8 ± 6.6	No significant changes
Proportion of hexagonal cells (%)	50.3 ± 8.2	49.3 ± 9.2	51.2 ± 8.6	$P = 0.03$ for post-SLT to 1 month
Central corneal thickness (μm)	536 ± 38	539 ± 41	537 ± 41	$P = 0.03$ for pre-SLT to post-SLT

SLT indicates selective laser trabeculoplasty.

TABLE 3. Corneal Cell Parameters Before and After Retinal Photocoagulation

	Pre-RP	Post-RP	1 Month	Significance
Endothelial cell density (cells/mm^2)	2559 ± 308	2549 ± 287	2549 ± 307	No significant changes
Coefficient of variance of cell area	38.1 ± 5.5	40.2 ± 6.8	39.4 ± 6.4	$P = 0.001$ for pre-RP to post-RP
Proportion of hexagonal cells (%)	50.6 ± 8.0	50.1 ± 7.3	50.4 ± 8.2	No significant changes
Central corneal thickness (μm)	541 ± 34	543 ± 35	543 ± 35	No significant changes

RP indicates retinal photocoagulation.

software. The coefficient of variance of cell area is a measure that quantifies polymegathism. It is calculated by dividing the standard deviation of the mean cell area by the value of mean cell area. Thus, a larger value implies a greater amount of cell size variability. The percentage of hexagonal cells is a measure quantifying pleomorphism. Although the normal corneal endothelium in young subjects has approximately 60% of its cells as hexagonal, stress to the corneal endothelium reduces the percentage of hexagonal cells.[9]

A repeated measures analysis of variance with nested design was used to test for differences at each time point for corneal endothelial cell density, CCT, coefficient of variance in cell size, and percentage of hexagonal cells. This design accounts for the lack of complete independence using both eyes from some patients.[18] The association of peak laser energy, total laser energy, and total laser shots with the aforementioned parameters was performed using Pearson correlations.

RESULTS

Patient Characteristics

There were 244 eyes from 133 patients who underwent SLT for open-angle glaucoma. Of these patients, 61 (45.8%) were men, and the mean age was 70.4 ± 11.4. A majority of the patients, 105 (78.9%), were of Asian ethnicity. Of the eyes included in the analysis, 123 (50.4%) were left eyes and 121 (49.6%) were right eyes. In the SLT cohort, the mean number of glaucoma eye drops used was 1.4 ± 0.9. The mean CCT was 536 ± 38 μm. For the SLT procedures, the mean number of laser shots was 61.1 ± 13.3 shots, the mean peak energy was 0.87 ± 0.24 mJ, and the mean total laser energy was 40.6 ± 17.4 mJ.

In the RP cohort, there were 132 eyes from 74 patients. The vast majority of patients (n = 72) received RP to treat retinal holes, tears, and degeneration, with the remaining 2 patients treated with RP for diabetic retinopathy. In the RP cohort, 36 (48.6%) were men, and the mean age was 64.7 ± 14.2. A majority of the patients, 61 (82.4%), were of Asian ethnicity. Of the eyes included in the analysis, 64 (48.5%) were left eyes and 68 (51.5%) were right eyes. The mean CCT was 541 ± 36 μm. Data regarding treatment parameters of the photocoagulation procedure was not collected as the information would not be useful due to the different retinal conditions treated.

SLT Cohort

The mean endothelial cell density pre-RP was 2292 ± 449 cells/mm^2, which decreased to 2265 ± 445 cells/mm^2 and increased slightly to 2279 ± 448 cells/mm^2 at 1 month. There was a statistically significant decrease between baseline and post-SLT ($P = 0.008$) and a statistically significant increase between post-SLT and 1 month ($P = 0.04$). Overall there was a statistically significant decrease between pre-SLT and at 1 month ($P = 0.04$).

The mean coefficient of variance of cell area was 38.5 ± 6.0 pre-SLT, 38.2 ± 6.9 post-SLT, and 38.8 ± 6.6 at 1 month. There were no significant differences between the means at any time points.

The proportion of hexagonal cells was 50.3 ± 8.2% pre-SLT, 49.3 ± 9.2% post-SLT, and 51.2 ± 8.6% at 1 month. There was no significant difference between pre-SLT and post-SLT ($P = 0.15$), but there was a significant increase between post-SLT and 1 month ($P = 0.03$).

The mean CCT was 536 ± 38 μm pre-SLT, and this increased to 539 ± 41 μm post-SLT and decreased to 537 ± 41 μm at 1 month. The increase between baseline and post-SLT was statistically significant ($P = 0.03$), however, there was no significant difference between baseline and 1 month ($P = 0.6$).

Total laser energy was not correlated with the change to the CCT immediately post-SLT ($P > 0.2$) but was correlated with CCT increase at 1 month (r = 0.26, $P = 0.01$). No other correlations between either total laser energy or peak laser energy and any quantitative or morphological parameters were significant.

Retinal Photocoagulation Cohort

The mean endothelial cell density pre-SLT was 2559 ± 308 cells/mm^2, which was unchanged at 2549 ± 287 cells/mm^2 post-RP and 2549 ± 307 cells/mm^2 at 1 month.

The mean coefficient of variance of cell area was 38.1 ± 5.5 pre-RP, 40.2 ± 6.8 post-RP, and 39.4 ± 6.4 at 1 month. There was a significant increase ($P = 0.001$) between pre-RP and post-RP but no change between post-RP and 1 month.

The proportion of hexagonal cells was 50.6 ± 8.0% pre-RP, 50.1 ± 7.3% post-RP, and 50.4 ± 8.2% at 1 month. There were no significant changes between any time points.

The mean CCT was 541 ± 34 μm pre-RP and was 543 ± 35 μm post-RP and 543 ± 35 μm at 1 month. There were no significant changes between any time points.

No patients in this cohort developed complications attributable to the RP procedure.

DISCUSSION

The present study found quantitative and morphological changes to the corneal endothelium after SLT that seem to begin recovering by 1 month. There was a small (just over 1%) but statistically significant reduction in corneal endothelial cell count from pre-SLT to post-SLT, and a statistically significant recovery at 1 month. Although polymegathism, as measured by the coefficient of variance in cell area, did not change, changes in pleomorphism were observed. The proportion of hexagonal cells did not change between pre-SLT and post-SLT, but there was a statistically significant 2% increase between post-SLT and 1 month. The only change of note in the RP group was an increase in polymegathism between pre-RP and post-RP.

SLT and RP are laser procedures commonly performed at a wavelength of 532 nm. In this study, mean laser energy for SLT was 40.6 ± 17.4 mJ, and although RP laser energy was not recorded individually, with settings of 200–400 mW and 300 μm spot size over 0.1 s duration, total energy delivered would be 15–30 mJ.[11] SLT uses a short 3 ns pulse, whereas RP uses a longer 0.1 s pulse. Thus, although both procedures use similar amounts of laser energy, it is possible that the delivery of this energy over a much shorter time period contributes to the greater degree of changes to the corneal endothelium after SLT observed here.

To the best of the authors' knowledge, this is the first cohort study looking at changes to the corneal endothelium after RP. Previously, argon laser photocoagulation (with a wavelength of 514 nm) had been found to decrease endothelial cell density, whereas in a contemporary cohort RP performed before vitrectomy protected against endothelial cell damage.[16,19] Although RP did not change endothelial cell density, it seemed to transiently increase polymegathism, suggesting that there may still be stress on the corneal endothelium. Longer term studies are needed to confirm this. It should also be noted that the RP cohort here had a higher baseline endothelial cell density, probably due in part to the younger average age. There were also very few RP procedures performed for diabetic retinopathy in this cohort, and thus further research is required in this group.

Quantitative changes to corneal endothelial cell density after SLT were similar to previous studies. Lee et al found a 5% decrease in cell count at 1 week that recovered (though not entirely to baseline levels) by 1 month.[14] The higher drop in cell count may be explained by the higher energy used in their SLT procedure, with mean total laser energy of 163 mJ compared with 40.6 mJ in the present study. Ong et al, in a previous study, found no significant change in cell count after SLT, though it is likely that study was underpowered to detect this relatively subtle change.[13]

Few morphological changes were observed, with no change from pre-SLT to post-SLT and only a slight increase in hexagonal cells from post-SLT to 1 month. The percentage of hexagonal cells is a measure of pleomorphism, and a healthy cornea will have approximately 60% of hexagonal cells.[9] A reduction in this value may indicate stress to endothelial cells. The present study may have been underpowered to detect a decrease between pre-SLT and post-SLT, though the increase by 1 month suggests an ongoing remodeling process. Although changes in polymegathism, represented by the coefficient of variance of cell area, were not noted here, polymegathism has been shown to increase in patients undergoing SLT for pseudoexfoliative glaucoma.[20]

CCT was transiently increased between pre-SLT and post-SLT. This is in contrast with the study by Lee et al, which found a transient decrease in CCT, whereas a cohort study by Guven Yilmaz et al found an increase in CCT persisting to 1 month post-SLT.[21] On one hand, SLT may cause endothelial cell dysfunction, leading to corneal edema and an increase in CCT. On the other hand, SLT may induce stromal contractions caused by the dissipation of heat energy from the laser, decreasing CCT.[14] It remains unclear which of these mechanisms is dominant, and may be influenced by other factors, such as Lee et al' use of topical nonsteroidal anti-inflammatory drugs for 1 day post-SLT.[14] Regardless, the present study concurs with Lee et al in the return to CCT baseline at 1 month, this may be due to stromal remodeling and corneal healing.[22]

Although clinically significant complications after SLT are rare, changes to the corneal endothelium have been documented. White et al noted dysmorphic cells and increased intracellular spacing on confocal microscopy after SLT.[23] They suggested this might be the result of endothelial cell swelling.

The mechanism for these changes is still not well understood. Leahy et al found decreased and irregular ZO-1 staining in cadaver eyes undergoing SLT, suggesting that endothelial tight junctions may be dysfunctional.[12] A previous study on rabbit eyes treated with SLT showed an increase in oxygen-free radicals in the aqueous humour after the procedure, and this may mediate this damage to the tight junctions.[24]

One limitation in the present study was that ideally the examiner of the specular microscopy image should have been blinded to whether the image was pre- or postprocedure. This was not done due to resource limitations, but may have introduced some measurement bias.

In conclusion, SLT causes a transient decrease in endothelial cell density and an increase in CCT, and there may be changes to pleomorphism, though this was only significant for the recovery between post-SLT and 1 month. RP seems to have significantly less impact on the corneal endothelium, with the only change of note an increase in polymegathism between pre-RP and post-RP. Overall the changes observed in the present study are minor and unlikely to be of clinical significance.

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