Long term effect of YAG laser iridotomy on corneal endothelium in primary angle closure suspects: a 72-month randomized control study

Running title: Effect of LPI on corneal endothelium.

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Abstract

Purposes: To evaluate the effect of YAG laser peripheral iridotomy (LPI) on corneal endothelial cell density (ECD) and morphological change in primary angle closure suspects (PACS) over 72 months.

Methods: The Zhongshan Angle Closure Prevention (ZAP) Trial is a single-center randomized controlled trial. Subjects with bilateral PACS were enrolled and received YAG LPI prophylactic treatment in one eye randomly, while the fellow eye served as control. Central corneal ECD and morphology were assessed bilaterally using non-contact specular microscopy (SP-2000P, Topcon, Tokyo, Japan) at baseline, 6, 18, 36, 54 and 72 months postoperatively. Mixed model analysis was conducted to compare the difference between treated and fellow eyes.

Results: A total of 875 participants with complete data were included in the analysis, with a mean age of 59.3±5.0 years and 83.5% female. The ECD declined significantly ($p<0.001$) over time in both treated and fellow eyes, but the treated eyes showed more progressive cell loss with increasing time ($p<0.001$). The difference in ECD loss from baseline between LPI treated and fellow eyes was not significant at each follow-up until 72 months (4.9% in LPI eyes vs. 4.2% in non-LPI eyes, $p=0.003$). Mean cell areas increased significantly over time in both treated and fellow eyes ($p<0.001$), but no longitudinal change was observed for hexagonality. In LPI treated eyes, no significant correlation was found between age, gender, ocular biometrics, intraocular pressure and laser settings with endothelium change, except for time effect ($p <0.01$).

Conclusion: ECD decreases over time primarily due to ageing effect. YAG LPI does not appear cause clinically significant corneal endothelial damage over 72 months after treatment.

Key words: laser peripheral iridotomy; safety; corneal endothelial cell; primary angle closure

Clinical trial registry: ISRCTN45213099.

Introduction

Primary angle closure glaucoma (PACG) accounts for nearly a half of global blindness caused by glaucoma.[1] Laser peripheral iridotomy (LPI) is the conventional first-line treatment for individuals with primary angle closure.[2] Previous evidence suggests that LPI can reduce elevated intraocular pressure (IOP) in primary angle closure (PAC) and reduce the risk of primary angle closure suspects (PACS) developing more PAC or acute attacks.[3, 4] While LPI is generally felt to be safe, there have been reports of corneal complications including edema and decompensation after argon laser iridotomies. [5, 6]

Compared with the photocoagulative thermal mechanism of an argon laser, Nd:YAG laser causes photo-disruption of target tissues with a high-power density, fewer spots of shorter duration and less energy for iris penetration. Though short-term safety of Nd:YAG LPI has been established, long-term evaluation is limited.[7] A previous study observed no significant change of endothelial cell count in 126 eyes underwent Nd:YAG LPI after 1 year.[8] Ramani et al.[9] also noted no significant change in central corneal thickness in 82 eyes 2 years after Nd:YAG LPI. However, corneal decompensation after LPI may be late-onset, evidenced by some studies reporting that bullous keratopathy or corneal edema occurred years after the LPI procedure.[5, 10]

The Zhongshan Angle Closure Prevention (ZAP) Trial is a single-center, randomized interventional controlled trial with the aims to evaluate the efficacy and safety of LPI for preventing PAC events in PACS subjects. The aim of the current analysis was to evaluate the influence of Nd:YAG LPI on corneal endothelial cell density and morphology in PACS patients over 72 months.

Methods

This trial was approved by the Ethical Review Board of Sun Yat-Sen University, the Ethical Committee of Zhongshan Ophthalmic Center, and the Moorfields Eye Hospital (via the London School of Hygiene & Tropical Medicine) and Johns Hopkins University institutional review boards. The study was conducted in accordance with the tenets of the Declaration of Helsinki. Study participants were recruited from a randomized controlled clinical trial, the ZAP trial (Trial registration ID: ISRCTN45213099). The International Standard Randomized Controlled Trial Number was issued on May 6, 2008. Written informed consent was obtained from all participants before enrolling.

Details of the sampling and recruitment methodology for this trial have been described previously.[11] In brief, participants aged 50–70 years from an urban district in Guangzhou diagnosed as bilateral PACS were enrolled. PACS was defined as the presence of 6 or more clock hours of angle circumference in which the posterior trabecular meshwork was not visible under static gonioscopy, with IOP equal to or less than 21 mmHg, absence of peripheral anterior synechiae or glaucomatous optic neuropathy, and no evidence of anterior segment ischemia from a previous acute IOP increase. Subjects with severe health problems, history of intraocular surgery or penetrating eye injury, media opacity preventing LPI, best corrected visual acuity worse than 20/40 or IOP increase greater than 15 mmHg after dilation or after a 15min dark room prone provocative testing were excluded. All eligible participants underwent prophylactic LPI in one randomly selected eye, while the fellow eye served as a control. The randomization was carried out with a pre-generated list of random numbers. Each eligible participant was assigned a number according to his/her sequence of entering the study. Randomization numbers and their corresponding eye assignment were generated at the data-monitoring center at Wilmer Eye Institute and sent to the clinical data-collection center at Zhongshan Ophthalmic Centre in sealed envelopes.

Comprehensive eye examinations were conducted on all eligible participants.[11] The IOP was measured using Goldmann applanation tonometry (Haag-Streit AG, Koeniz, Switzerland). The median of 3 readings for each eye was considered. Ocular biometric measures such as axial length (AL), central anterior chamber depth (ACD), and lens thickness (LT) were acquired using ultrasound A-scan (CineScan A/B, Quantel Medical, Bozeman, MT). All subjects underwent central corneal endothelium assessment bilaterally using a non-contact, semi-automated specular microscope (SP-2000P, Topcon, Tokyo, Japan). This instrument can capture a digital image of the corneal endothelium and automatedly calculate the number of cells per mm^2 (endothelial cell density, ECD), average cell size and percentage of hexagonal cells in the area analyzed (hexagonality, %). Endothelial cell density was recorded to track the cell loss. The average cell size was used to measure the extent of variation in cell area (polymegathism) while the hexagonality was used as an index of variation in cell shape (pleomorphism). Baseline examination was conducted before LPI and at 6, 18, 36, 54 and 72 months postoperatively.

Fifteen minutes after the administration of 2% pilocarpine and 0.15% brimonidine eye drops, LPI was performed using YAG laser (Visulas YAG III, Carl Zeiss Meditec, Dublin, CA, USA) with the use of an Abraham lens (Ocular Instruments, Bellevue, WA, USA). The laser irradiated the iris with an initial setting of 1.5 mJ increasing as needed to create a patent iridotomy of at least 200 μm in diameter. The LPI was placed in a crypt or other area where the iris appeared thinnest and was positioned beneath the superior lid. The IOP was checked 1 hour postoperatively, with pressure-lowering treatment administered as needed. All participants were prescribed dexamethasone drops applied hourly for 24 hours after surgery and then 4 times daily for 1 week.

Statistical Analysis

Differences in baseline characteristics between the LPI and non-LPI groups were assessed with standard parametric tests (t test) if data were normally distributed and nonparametric tests (Mann-Whitney test) if the data were not normally distributed. Changes in endothelial cell parameters were compared between the LPI and non-LPI groups using mixed-effects models. Time after operation was set as the time variable and calculated per month. Unstructured covariance structure was selected after comparing other covariate structures, such as the variance component, compound symmetry, first order autoregressive, and Toeplitz, based on the smallest Akaike information criterion and Bayesian information criterion values. All of the model covariates were adjusted for age at baseline and gender. Group assignment, follow-up time, and group × time interactions were included as fixed effects. Mean changes and 95% confidence intervals derived from the mixed models were calculated. P values <0.05 were considered significant. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc).

Results

Of the 889 eligible participants enrolled at baseline, 875 participants with complete data were included in the analysis. The mean age was 59.3±5.0 years and 83.5% were women (n=875). Twelve participants had repeat LPI to complete the iridotomy and 15 participants had LPI in the control eye before the last follow-up examination. The baseline characteristics of treated eyes and controls eyes are displayed in Table 1. No significant difference in specular biomicroscopy parameters was observed between treated and fellow eyes at baseline (all with $p>0.05$).

ECD decline over time in eyes with or without LPI treatment (Table 2). The difference in ECD between LPI treated and fellow eyes was not significant at each follow-up until 72 months after operation. The mean percent reduction of ECD from the preoperative to the 72-month postoperative was 4.93% (95% CI: -5.3%, -4.57%) in LPI-treated eyes and 4.2% (95% CI: -4.57%, -3.83%) in untreated eyes, respectively (p=0.003). Additionally, the mean cell area and the hexagonality of cells increased in both eyes, but the differences were not significant between groups over time, except for the mean cell area measurement at 54-month follow-up (p=0.03).

Mixed model analysis was conducted to investigate risk factors for longitudinal changes in corneal endothelial cells parameters (Table 3). Baseline age was significantly negatively associated with ECD level, as older patients had greater endothelial cell loss at each timepoint (p=0.001). The cell density declined significantly (p<0.001) over time in both treated and fellow eyes, but the month to month endothelial cell loss was on average 0.32 per mm^2 greater for the LPI group (p<0.001). Risk factors for mean cell area change mirrored those for ECD change, as older patients presented larger mean cell area at each timepoint (p=0.007) and the difference between groups enlarged over time (p=0.008). However, no significant correlation was found between above risk factors and hexagonality of endothelial cells (with all p>0.18).

Table 4 demonstrates the risk factors relating to the longitudinal changes of corneal endothelial cells parameters after receiving YAG LPI treatment. Age, gender, ocular biometrics, IOP and laser settings did not affect ECD decline and morphological change in mean cell area and hexagonality (with all p>0.05). Time since LPI is the main factor associated with the longitudinal changes in endothelial cells parameters (with all p <0.01).

Discussion

Over 72 months of observation after YAG LPI, corneal endothelial cell density declined and morphology changed in both treated and fellow eyes of PACS patients. Though the difference in endothelial cell density between treated and fellow eyes was not statistically significant before 54 months, it became statistically significant at 72 months after LPI. That said, the difference was small, less than 1%, and likely has no clinical impact.

The impact of YAG LPI on the corneal endothelium has been reported to be both safe and harmful in previous studies. Wishart et al.[12] reported no significant change in ECD of patients who received YAG LPI, but observed two cases of local endothelial changes. Other studies have documented a significant decline in ECD after YAG LPI without comparison to a control group.[13, 14] As for longer-term effect, several prospective studies have not found any significant change in endothelial cell counts one year after YAG LPI.[8, 15, 16] However, Wu et al.[17] reported a significant endothelial cell lost (7.0%, $p<0.001$) in 1-year follow-up, which was much higher than that in our study (0.97% at 18 months). The significant loss of endothelial cell may be attributed to the variance of laser energy settings (total energy ranged 22.4 to 250.3 mJ) and methods for calculating ECD (average of photographs of central and 4 peripheral quadrants). The current study is consistent with these previous findings of minimal impact over the short-term.

In contrast to the current findings, one retrospective study reported no significant change in the ECD in the central cornea in patients who had YAG LPI performed 1-15years previously.[18] The current study found no association between ECD loss and laser energy required to complete the LPI, suggesting the energy used to create the LPI was not the primary cause of the long-term effect and was minimally invasive to endothelial cells. It is possible that endothelial cell damage could result from environmental changes at the level of the endothelium after iridotomy owing to alterations in aqueous dynamics, breakdown of the blood-aqueous barrier and suspended debris in the aqueous humor,[19-21] since these risk factors can persist after the procedure. Although the difference seen in the current study are not clinically significant and no case of corneal decompensation was observed during the follow-up, whether the difference between treated and control eyes will continue to increase beyond 72 months remains unknown.

Ageing is another important factor accounting for the ECD decline in eyes with or without YAG LPI. Previous studies shown that normal eyes lose 0.25% to 1% of the endothelial cells each year (Table 5). The rate in untreated eyes in our study [0.93% (95%CI, -1.26, -0.6) in 18 months] was slightly higher than that in normal eyes reported in most previous studies although a direct comparison may not be appropriate when ethnicity and demography of the participants and methods on ECD measurement are substantially different. One possible explanation is that eyes with shallower anterior chambers are more predisposed to endothelial cell loss due to the increased possibility of irido-trabecular contact. Varadaraj et al[22] is the

only study reporting that angle closure suspects have lower ECD than those with open angles although controversially they reported established primary angle closure glaucoma had similar level of ECD as the open angle eyes.[22] In another cohort of PACS subjects, the mean endothelial loss was 3.2% at 1 year and 0.9% at 3 years in PACS eyes without LPI,[23] raising some concerns about the validity of data in this study. Correspondingly, the mean endothelial cell loss in our study was 0.93% at 18 months and 2.38% at 3 years. Given the fact that we did not find that corneal endothelial cell count was associated with anterior chamber depth at baseline (correlation coefficient=-0.01, p=0.55), we conclude that the ECD changes over time among the untreated eyes is primarily driven by ageing effects instead of narrow angles.

The randomized control trial design, large sample size, high follow-up rate, long duration of observation and mixed model analysis allowing for inclusion of a time effect are the major advantages of this study. However, we only observed the central corneal epithelium and the examination might not be in the exact same area of the cornea at every visit. Therefore, focal damage and differences in the peripheral cornea may have been missed. Further investigation of the peripheral cornea would help to understand better the long-term safety of YAG LPI.

Conclusions

In summary, a significant decline in ECD was observed in both YAG LPI-treated eyes and control eyes, which was primarily attributed to the ageing effect. The difference between them was not statistically significant until 72 months after the procedure, with a smaller difference of 0.74% (95% CI, -1.23% to -0.24%) and minimum clinical significance.

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Table1. Baseline characteristics of treated and fellow eyes (n=875).

Characteristics	**LPI**	**Non-LPI**	**P-value**
Age (years)	59.3±5.0		-
Female (%)	731 (82.9)		-
Endothelial cell density (per mm^2)	2571.4±300.3	2571.9±304.9	0.94
Mean cell area (μm^2)	394.9±53.9	395.0±54.0	0.96
Hexagonality (%)	56.5±9.4	56.9±9.4	0.38

LPI=laser peripheral iridotomy

Table 2. Changes of corneal endothelial cell parameters for YAG laser peripheral iridotomy treated and fellow eyes at each follow-up.

Month	Absolute change [Mean (95% CI)]			Percentage change [%, (95% CI)]			P-Value
	LPI eye	Non-LPI eye	Treatment effect	LPI eye	Non-LPI eye	Treatment effect	
Endothelial cell density (per mm^2)							
6	-11.8 (-20.2, -3.3)	-20.3 (-28.8, -11.9)	8.6 (-2.9, 20.1)	-0.39 (-0.72, -0.06)	-0.65 (-0.98, -0.32)	0.26 (-0.19, 0.71)	0.14
18	-25.4 (-33.8, -17)	-26.4 (-34.8, -17.9)	1.0 (-10.5, 12.4)	-0.97 (-1.3, -0.63)	-0.93 (-1.26, -0.6)	-0.03 (-0.48, 0.41)	0.87
36	-65.5 (-74.2, -56.9)	-62.4 (-71.1, -53.7)	-3.1 (-14.9, 8.7)	-2.5 (-2.84, -2.16)	-2.38 (-2.72, -2.04)	-0.12 (-0.58, 0.34)	0.61
54	-117.9 (-126.9, -108.9)	-106.3 (-115.3, -97.2)	-11.6 (-23.9, 0.7)	-4.56 (-4.92, -4.21)	-4.08 (-4.44, -3.73)	-0.48 (-0.96, 0)	0.06
72	-127.8 (-137, -118.6)	-108.4 (-117.8, -99)	-19.4 (-32.1, -6.7)	-4.93 (-5.3, -4.57)	-4.2 (-4.57, -3.83)	-0.74 (-1.23, -0.24)	**0.003**
Mean cell area (μm^2)							
6	1.1 (-0.2, 2.3)	1.8 (0.6, 3.1)	-0.8 (-2.5, 0.9)	0.35 (0.05, 0.66)	0.6 (0.29, 0.9)	-0.24 (-0.67, 0.18)	0.38
18	3.5 (2.2, 4.7)	3.7 (2.4, 4.9)	-0.2 (-1.9, 1.5)	0.94 (0.63, 1.25)	1.04 (0.73, 1.35)	-0.1 (-0.53, 0.33)	0.81
36	9.0 (7.7, 10.3)	9.3 (8, 10.5)	-0.3 (-2, 1.5)	2.4 (2.08, 2.72)	2.43 (2.12, 2.75)	-0.03 (-0.47, 0.4)	0.75
54	17.3 (16, 18.6)	15.2 (13.9, 16.6)	2.1 (0.2, 3.9)	4.48 (4.15, 4.8)	3.99 (3.66, 4.33)	0.48 (0.02, 0.94)	**0.03**
72	16.5 (15.2, 17.9)	15.1 (13.7, 16.4)	1.5 (-0.4, 3.4)	4.32 (3.98, 4.66)	3.91 (3.57, 4.26)	0.4 (-0.07, 0.88)	0.13
Hexagonality (%)							
6	0.16 (-0.48, 0.80)	0.15 (-0.49, 0.80)	0 (-0.88, 0.89)	2.19 (0.93, 3.45)	2.31 (1.05, 3.58)	-0.13 (-1.85, 1.59)	0.99
18	0.06 (-0.58, 0.70)	0.28 (-0.37, 0.93)	-0.22 (-1.11, 0.67)	2.15 (0.89, 3.42)	2.66 (1.39, 3.93)	-0.5 (-2.23, 1.23)	0.63
36	0.12 (-0.54, 0.77)	0.07 (-0.59, 0.73)	0.05 (-0.86, 0.96)	2.24 (0.95, 3.53)	2.2 (0.9, 3.49)	0.04 (-1.72, 1.81)	0.92
54	1.20 (0.52, 1.88)	0.37 (-0.31, 1.06)	0.83 (-0.11, 1.78)	4.16 (2.83, 5.49)	2.83 (1.49, 4.18)	1.33 (-0.51, 3.16)	0.08
72	0.53 (-0.16, 1.22)	0.40 (-0.31, 1.11)	0.13 (-0.84, 1.10)	2.89 (1.53, 4.25)	3.08 (1.69, 4.47)	-0.19 (-2.07, 1.7)	0.80

LPI=laser peripheral iridotomy; 95%CI=95% confidential interval.

Treatment effect defined as the change in the LPI group minus the change in the control group. P-value for the differences between groups.

Bold indicates statistical significance.

Table 3. Mixed model estimates of risk factors for longitudinal crude changes of corneal endothelial cells parameters over 72 months.

Multivariate models	Estimate (95%CI)	P-value
Endothelial cell density (per mm^2)		
Age at baseline (years)	-1.45 (-2.32, -0.58)	**0.001**
Gender (Male as reference)	-5.27 (-16.77, 6.24)	0.37
Group (Non-LPI eye as reference)	6.07 (-2.46, 14.61)	0.16
Time (Month)	-1.60 (-1.72, -1.48)	**<0.001**
Time × Group	-0.32 (-0.49, -0.15)	**0.0002**
Mean cell area (μm^2)		
Age at baseline (years)	0.18 (0.05, 0.31)	**0.007**
Gender (Male as reference)	1.21 (-0.51, 2.93)	0.17
Group (Non-LPI eye as reference)	-0.68 (-2.01, 0.65)	0.32
Time (Month)	0.23 (0.22, 0.25)	**<0.001**
Time × Group	0.03 (0.01, 0.06)	**0.008**
Hexagonality (%)		
Age at baseline (years)	0 (-0.07, 0.07)	0.98
Gender (Male as reference)	0.62 (-0.30, 1.54)	0.18
Group (Non-LPI eye as reference)	-0.07 (-0.77, 0.63)	0.85
Time (Month)	0 (0, 0.01)	0.28
Time × Group	0.01 (-0.01, 0.02)	0.28

LPI=laser peripheral iridotomy; 95%CI=95% confidential interval.

Time × Group= interaction between time and group

Bold indicates statistical significance.

Table 4. Mixed model estimates of baseline factors for longitudinal crude changes of corneal endothelial cells parameters after laser peripheral iridotomy over 72 months.

Predictors	**Endothelial cell density (mm^2)**		**Mean cell area (μm^2)**		**Hexagonality (%)**	
	Estimate (95%CI)	**P-value**	**Estimate (95%CI)**	**P-value**	**Estimate (95%CI)**	**P-value**
Age at baseline (years)	-1.91 (-6.03, 2.21)	0.36	0.2 (-0.54, 0.95)	0.59	-0.08 (-0.17, 0.01)	0.09
Gender (Male as reference)	-33.92 (-88.31, 20.47)	0.22	0.74 (-9.09, 10.56)	0.88	-0.47 (-1.68, 0.75)	0.45
Time (Month)	-1.91 (-2.03, -1.78)	**<0.001**	0.27 (0.25, 0.29)	**<0.001**	0.01 (0, 0.02)	**0.01**
Axial length at baseline (mm)	-3.6 (-33.2, 26.01)	0.81	0.31 (-5.01, 5.63)	0.91	0.2 (-0.47, 0.87)	0.56
ACD at baseline (mm)	4.97 (-93.42, 103.36)	0.92	-1.03 (-18.24, 16.19)	0.91	-0.82 (-3.16, 1.53)	0.50
Lens thickness at baseline (mm)	3.72 (-57.5, 64.94)	0.91	-2.04 (-12.66, 8.57)	0.71	-1.29 (-2.77, 0.19)	0.09
IOP (mmHg)	-0.02 (-0.2, 0.15)	0.78	0 (-0.02, 0.03)	0.88	0 (-0.02, 0.01)	0.57
Laser Number of shots (n)	-0.34 (-0.86, 0.17)	0.19	0.07 (-0.02, 0.16)	0.13	0 (-0.01, 0.01)	0.80
Laser energy Power (mJ)	-13.02 (-26.34, 0.3)	0.06	2.2 (-0.05, 4.45)	0.06	0.12 (-0.24, 0.47)	0.52
Total quantity of laser energy (mJ)	0.05 (-0.18, 0.29)	0.66	0 (-0.04, 0.04)	0.90	0 (-0.01, 0)	0.46

IOP=intraocular pressure; ACD= Anterior chamber depth.

Bold indicates statistical significance.

Table 5. Annual change of corneal endothelial cell density (ECD) in normal eyes.

Year	Study site	Study type	Number of eyes/subjects	Age (years)	Follow-up (years)	ECD change/year
1985[24]	UK	Longitudinal	103 eyes	11 participants were <65; 53 were between 65 and 74; and 39 were ≥75 years	2	-1%
1985[25]	UK	Longitudinal	48 subjects	Not mentioned	2	-0.6%
1993[26]	USA	Longitudinal	5 eyes	69.3 ± 8	5	-0.80%
1993[27]	Japan	Longitudinal	9 subjects	67.4 ± 7.7	5	-0.26% ± 1.32% (-8.36 to 17.4 cells/mm^2)
1997[28]	USA	Longitudinal	42 subjects	59.5 ± 16.8 (range 30-84, at the recent follow-up)	10.6 ± 0.2	-0.6% ± 0.5% (-16 cells/mm^2)
1985[29]	USA	Cross-sectional	60 eyes	Range 12-85	-	-9.45 cells/mm^2
2000[30]	India	Cross-sectional	537 eyes	48 ± 16.5 (range 20-87)	-	-0.30%
2006[31]	Iran	Cross-sectional	525 eyes	52.7 ± 19.1 (range 20-85)	-	-0.60%
2007[32]	China	Cross-sectional	1329 eyes	44 ± 21 (range 10-98)	-	-0.30%
2007[33]	Auckland	Cross-sectional	85 subjects	38 ± 16 (range 18-87)	-	-0.50%
2010[34]	Japan	Cross-sectional	2602 eyes	59.1 ± 14.9	-	-0.25% (-7.43 cells/mm^2)
2017[35]	Pakistan	Cross-sectional	464 eyes	39.52 ± 18.09 (range 12-80)	-	-0.28%
2019[36]	Egypt	Cross-sectional	568 eyes	49 ± 15.2 (range 20-85)	-	-0.30%