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Autologous serum therapy in recalcitrant laser-assisted in situ keratomileusis-induced neurotrophic epitheliopathy

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ABSTRACT

Background/Purpose: To evaluate the efficacy of autologous serum eye drops for patients with recalcitrant laser-assisted in situ keratomileusis (LASIK)-induced neurotrophic epitheliopathy (LINE) unresponsive to conventional treatment, and to determine the possible predisposing risk factors of these patients.

Methods: We enrolled 10 consecutive patients (20 eyes) undergoing femtosecond-assisted myopic LASIK surgery presenting with recalcitrant LINE for > 1 year. Another 340 patients (713 eyes) receiving femtosecond-assisted myopic LASIK without recalcitrant LINE were set as controls. Possible risk factors associated with recalcitrant LINE were investigated. Twenty percent autologous serum treatment was prescribed to 20 eyes. The efficacy of autologous serum was assessed with ocular surface conditions, tear function, and the change of best-corrected visual acuity.

Results: Age older than 30 years [odds ratio (OR) = 7.74; 95% confidence interval (CI), 1.74–34.50], flap thickness < 110 μm (OR = 3.47; 95% CI, 1.22–9.73), and a flap diameter < 8.5 mm (OR = 5.38; 95% CI, 1.95–14.85) pose higher risks in femtosecond laser-assisted myopic LASIK. All eyes (100%) achieved remission after autologous serum treatment. The visual acuity before treatment was 0.49 ± 0.41 in LogMAR, and the visual acuity after treatment was 0.14 ± 0.22 in LogMAR. Time to achieve remission was 8.26 ± 11.87 weeks. Mean relapse-free survival after discontinuing autologous serum was 47 weeks.

Conclusion: Risk factors of recalcitrant LINE in femtosecond laser-assisted myopic LASIK were identified as older age, a thinner flap (<110 μm), and a small flap diameter (<8.5 mm). Autologous serum eye drops can effectively improve corneal surface conditions and postoperative visual acuity.

1. Introduction

Laser-assisted in situ keratomileusis (LASIK) is the most performed procedure for treating refractive errors.[1] For both microkeratome- or femtosecond-assisted LASIK, the standard procedure includes the creation of a flap with intact epithelium and ablation of the stromal surface with excimer laser keratomileusis. However, the creation of corneal flaps inevitably damages the nerve fibers within the flap, interrupting the cornea-trigeminal nerve-brainstem-facial nerve-lacrimal gland reflex arc which influences both basal and stimulated tear secretion and drives the blinking mechanism.[2] Occasionally, such interruption will result in LASIK-induced neurotrophic epitheliopathy (LINE).[3,4]

LINE always presents as a dry eye-like syndrome, which leads to the breakdown of the corneal epithelium a few days to weeks after surgery, and significantly impacts clinical outcomes and satisfaction.[3,4] Multiple risk factors have been implicated in LINE, including decreased neurotrophic factors to epithelial cells, reduced blinking

Conflicts of interest: The authors declare that they have no competing interests.

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rate, decreased reflex and basal tear production, change in tear film distribution, more evaporative tear loss, and possible damage to limbal goblet cells by the microkeratome suction ring.[4] Among these factors, the components in tears, including the neurotrophic factors that help nerve regeneration, epidermal growth factor, and vitamin A, play important roles in normal epithelialization.[5] When these components are depleted, epithelialization is impaired, leading to epithelial break down. According to its pathophysiology, it is reasonable to treat LINE with autologous serum.[5] Autologous serum was reported to be beneficial in keratoconjunctivitis sicca and persistent epithelial defects.[6,7] Matsumoto et al[3] demonstrated the promising effect of 20% autologous serum for the restoration of the ocular surface epithelial integrity in patients with neurotrophic keratopathy. Autologous serum harbors essential elements in the ocular surface: (1) neurotrophic mediators, such as insulin-like growth factor 1, nerve growth factor, and substance P; (2) components maintaining healthy epithelium, including epidermal growth factor, transforming growth factors (TGFs), and vitamin A[3,5]; and (3) anti-inflammatory factors and matrix metalloproteinase inhibitors, which can promote a greater epithelial healing rate.[3,5] Conventional treatments, such as artificial tears, alleviate tear insufficiency and solve most cases of LINE. However, recalcitrant cases with prolonged nerve regeneration exhibit a poor clinical response to conventional treatments and may benefit from essential elements of autologous serum. Herein, the purpose of this study is to investigate the predisposing risk factors of recalcitrant LINE and to evaluate the efficacy of autologous serum eye drops for patients with recalcitrant LINE.

2. Methods

2.1. Patients

We performed a retrospective review of 10 consecutive patients who visited the outpatient clinic at the Department of Ophthalmology, National Taiwan University Hospital, Taipei, Taiwan from January 2008 to May 2012. All 10 patients (20 eyes) with previous myopic LASIK surgery had recalcitrant LINE for > 1 year and failed to respond to conventional treatments such as artificial tears or punctal occlusion as described previously.[4] External eye conditions such as punctate keratitis and epithelial defects were documented with photography in every case as part of our daily practice (Fig. 1). Decreased corneal sensitivity was confirmed with quantitated corneal sensation measurement utilizing the Cochet-Bonnet esthesiometer. All patients were referred to our hospital by one local clinic (Nobel Eye Clinic, Taipei, Taiwan). Another 340 patients who received LASIK surgery in the same clinic without recalcitrant LINE were selected as the control group. There were 713 eyes, in which 33 eyes were retreated once. The eligible inclusion criteria included patients without presentations of LINE after surgery >1 year later and patients with a diagnosis of LINE improved with conventional treatments. Patients presenting systemic or ocular conditions that could interfere with ocular surface status were excluded. The research protocol was approved by the Human Research and Ethics Committee of the National Taiwan University Hospital, and informed consent for participation in the study was obtained from participants. Research carried out was in compliance with the Helsinki Declaration.

2.2. Preparation of autologous serum eye drops

Briefly, autologous serum was prepared from 30 mL of blood taken using venipuncture and centrifuged for 6 minutes at 3207 g in sterilized tubes. The separated serum was filtered and extracted. Two mL of serum were allocated to 10 mL bottles and diluted to 20% concentration by adding 8 mL sterile saline solution to each bottle. Six bottles were made at one time. Patients were instructed to keep the currently used bottle <4°C and other unused bottles <−20°C. The bottles were discarded if stored for >7 days.[3]

2.3. Examinations

The severity of neurotrophic epitheliopathy was evaluated and recorded from two aspects: poor ocular surface condition and tear dysfunction. Ocular surface conditions such as inflammation, fluorescein, and rose bengal staining on conjunctiva and cornea, and re-epithelialization were featured. All patients visited our outpatient clinic biweekly until re-epithelialization of the cornea without fluorescein stain had occurred. Tear function evaluations were incorporated into tear break-up time test, tear meniscus height, and Schirmer's test 1 with anesthesia at the first visit before treatment in all cases.

The pretreatment visual acuity was measured at the initial visit. The post-treatment visual acuity was recorded 2–4 weeks after autologous serum treatment. The efficacy of the autologous serum was evaluated by healing of neurotrophic epitheliopathy and the improvement of visual acuity in logMAR. The investigated factors associated with recalcitrant LINE included age, gender, the value of basal Schirmer's test, and spherical equivalent refraction (SER) correction in LASIK, flap size, and flap thickness. All eyes received myopic LASIK with femtosecond laser-created flaps (IntraLase; IntraLase Corp/Advanced Medical Optics Inc., Santa Anna, CA, USA).

2.4. Statistical analysis

To examine the significance of the investigated factors predisposing to neurotrophic epitheliopathy, binary logistic regression was examined to further verify the correlations of Schirmer's test, SER, flap size, and flap thickness documented before LASIK surgery with LINE. Remission of recalcitrant LINE was defined as complete re-epithelialization without fluorescein staining of the cornea. Once patients obtained remission, autologous serum was discontinued and shifted to conventional treatment. Kaplan–Meier survival curve for relapse of LINE was calculated in patients obtaining remission status. All statistical analyses were conducted using SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant.

3. Results

Multiple factors were investigated for their association with recalcitrant LINE: age, gender, tear function, and SER documented before LASIK surgery, flap size, and flap thickness. Table 1 demonstrates the basic characteristics of patients with recalcitrant LINE and patients without recalcitrant LINE. The logistic regression model assumes that the effects of the risk factors are multiplicative. The effect of each risk factor is expressed as an odds ratio (OR) for the development of recalcitrant LINE. According to the model, older patients (age > 30 years) were more vulnerable to recalcitrant LINE than young patients (age < 30 years), with an OR of 7.74 [95% confidence interval (CI), 1.74–34.50]. Femtosecond-created flaps with a thickness of 95–109 μm were more in danger than a thicker flap (110–130 μm), with an OR of 3.47 (95% CI, 1.22–9.73). A small flap diameter (8–8.4 mm) was also at higher risk than large flaps (8.5–9 mm), with an OR of 5.38 (95% CI, 1.95–14.85). Table 2 displays multivariate logistic regression of the possible factors correlated with recalcitrant LINE.

Autologous serum eye drops were prescribed for 20 eyes of 10 patients with recalcitrant LINE. The pretreatment visual acuity was 0.49 ± 0.41 LogMAR units, and the post-treatment visual acuity was

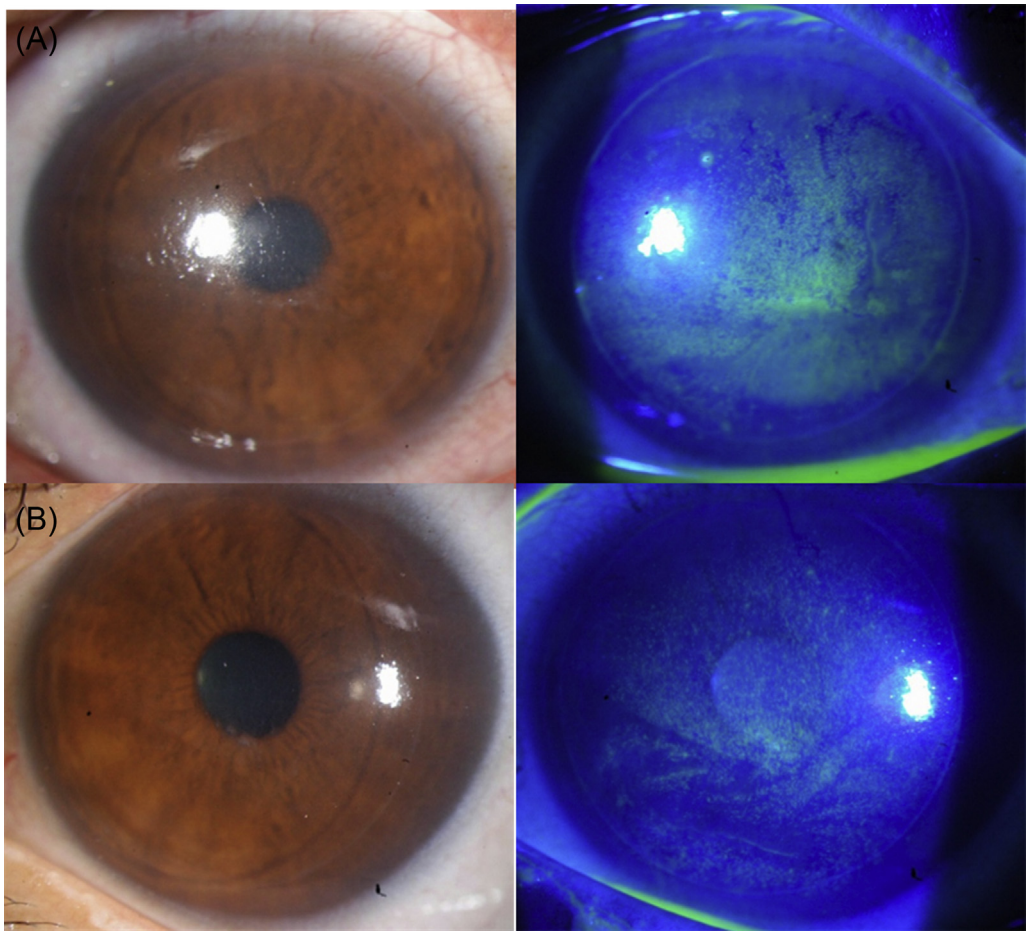

Fig. 1. External photography of one case with bilateral recalcitrant laser-assisted in situ keratomileusis-induced neurotrophic epitheliopathy External photography shows low tear meniscus and a classic fluorescein staining pattern with nearly confluent punctate epithelial erosions on over the laser-assisted in situ keratomileusis flap on (A) right eye and (B) left eye.

0.14 ± 0.22 LogMAR units. The improvement of visual acuity was 0.35 ± 0.33 LogMAR units. All eyes (100%) achieved remission after autologous serum treatment. Time to achieve remission was 8.26 ± 11.87 weeks. Eleven eyes (55%) received autologous serum treatment more than once. Kaplan–Meier survival curve analysis of LINE relapse after discontinuing autologous serum is illustrated in Fig. 2. Mean relapse-free survival was 47 weeks, and relapse-free probabilities at 10 weeks, 34 weeks, and 41 weeks were 86%, 78%, and 58%, respectively. The Schirmer's test value before treatment was 7.00 ± 3.77 mm, and the Schirmer's test value after treatment was 7.05 ± 2.38 mm.

4. Discussion

Post-LASIK tear dysfunction and dysesthesia syndrome is a spectrum of disease characterized as transient or persistent post-operative neurotrophic disease due to corneal denervation. Transection of the sub-basal nerve plexus and deep stromal nerve during flap creation are contributory to the main pathophysiology.[8] Although corneal sensation can return to normal value from weeks to months,[4] sub-basal nerve fiber density has been reported not to return to preoperative levels by 2 years in confocal microscopy studies.[9] Prolonged corneal nerve regeneration is to blame in

Table 1
Basic characteristics of patients with recalcitrant LINE and patients without recalcitrant LINE.

	With recalcitrant LINE (n = 20)	Without recalcitrant LINE (n = 713)
Gender (Female/male)	16/4	544/169
Age (y)	36.7 ± 4.96	32.22 ± 5.95
Before LASIK		
Schirmer's test (mm)	7.00 ± 3.77	7.79 ± 5.19
SER	7.12 ± 2.16	6.12 ± 1.86
Flap size (mm)	8.45 ± 0.56	8.71 ± 0.23
Flap thickness (μm)	103.50 ± 10.53	107.19 ± 5.56

LASIK = laser-assisted in situ keratomileusis; LINE = laser-assisted *in situ* keratomileusis-induced neurotrophic epitheliopathy; n = eye number; SER = spherical equivalent refraction.

Table 2
Multivariate logistic regression of the possible factors correlated with recalcitrant LINE.

Factors	Odds ratio	95% Confidence interval	*p*
Age (y)			
≤ 30	1		
> 30	**7.74**	1.74–34.50	**0.007**
Gender			
Female	1		
Male	0.76	0.23–2.59	0.662
Schirmer's test before LASIK (mm)	0.98	0.88–1.09	0.668
SER (diopter)	0.80	0.62–1.04	0.094
Flap-thickness (μm)			
110–130	1		
95–109	**3.47**	1.22–9.73	**0.02**
Flap-diameter (mm)			
8.5–9	1		
8–8.4	**5.38**	1.95–14.85	**0.001**

LASIK = laser-assisted in situ keratomileusis; SER = spherical equivalent refraction.
Bold represents significant values.

recalcitrant LINE. Several risk factors, such as patient demographics and flap characteristics, have been proposed but remained inconclusive in nerve regeneration after LSAIK. In this study, we found that patients older than 30 years, flap thickness <110 μm, and a flap diameter <8.5 mm pose higher risks in femtosecond laser-assisted myopic LASIK.

Several preoperative risk factors have been proposed, including Schirmer's test score, long-term contact lens wear, age, and female gender. Aging and female gender have both been found as risk factors in dry eye syndrome, but remained inconclusive in post-LASIK tear dysfunction.[10–12] In our study, older age was a risk factor in the development of recalcitrant LINE, especially in those older than 30 years. Female gender comprised 76% of patients receiving myopic LASIK in this study, but was not a risk factor in recalcitrant LINE. Some studies found that lower preoperative Schirmer scores are associated with tear dysfunction after LASIK. However, these studies focused on dry eye symptoms within 1 year.[12,13] Preoperative Schirmer's test value was not related to poor nerve regeneration after 1 year and recalcitrant LINE in this study.

Whether a thinner flap results in less postoperative neuropathic disease is hotly debated. Salomão and Wilson[14] found that flaps performed with a microkeratome (180 μm) had a significantly higher incidence of LINE, compared with femtosecond created flaps (110–120 μm). Conversely, Patel et al[15] showed that sub-basal nerve density and corneal sensitivity were similar in flaps created with microkeratome (180 μm) and femtosecond (120 μm) at any examination. In our study, all cases received flaps with femtosecond creation. We found those with a flap thickness <110 μm carry a significantly higher risk for the development of recalcitrant LINE than flaps between 110 μm and 130 μm ($p = 0.02$), implying slower nerve regeneration in this subgroup. Photodisruption of femtosecond laser at the level of Bowman's layers may be responsible for this phenomenon.

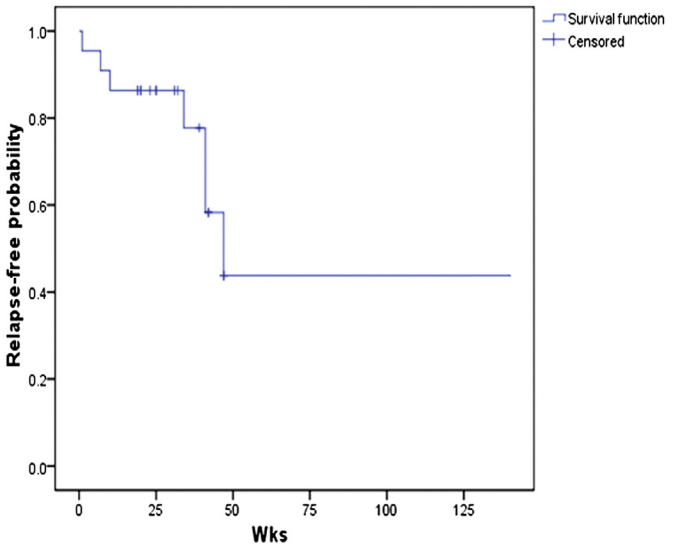

Fig. 2. Kaplan–Meier survival curve of laser-assisted in situ keratomileusis-induced neurotrophic epitheliopathy relapse after discontinuing autologous serum. Mean relapse-free survival was 47 weeks, and relapse-free probabilities at 10 weeks, 34 weeks, and 41 weeks were 86%, 78%, and 58%, respectively.

Flap hinge and its influence on corneal nerve damage during LASIK is also of interest. Muller et al[2] portrayed the main trunk of nerve entering the cornea at the 3 o'clock and 9 o'clock positions, then spreading centrally and anteriorly. They suggested a superiorly hinged corneal flap can transect less major corneal innervation than nasally hinged flap.[16,17] However, other studies illustrated nerve bundles entering the cornea at all directions.[18–20] The results of hinge positions and post-LASIK tear dysfunction were still conflicting. Larger hinge width was hypothesized to reserve more nerve bundle and reduce postoperative tear dysfunction.[21] All our cases received superiorly hinged flaps. Smaller flap diameter was correlated with smaller hinge width, and thus a higher risk of recalcitrant LINE, which was consistent with previous studies.

Loss of trophic influence on corneal epithelium and prolonged tear insufficiency as a consequence of poor reinnervation after LASIK results in ocular surface changes, namely LINE. Conventional treatments, such as artificial tears or punctal occlusion, alleviate tear insufficiency and solve most cases of LINE. However, for those intractable cases, trophic factors play a more important role in rebuilding healthy corneal epithelium. The effectiveness of autologous serum has been reported in the prolongation of the tear break up time and a reduction in rose bengal staining score 1 month, 3 months, and 6 months after LASIK.[22] In this study, we evaluated the efficacy of autologous serum treatment in patients with recalcitrant LINE with ocular surface condition and tear function. We opted for 20% autologous serum eye drops instead of other concentrations that were previously used in literature[23,24] based on the concentration of TGF-beta in tears. TGF-beta, which is believed to inhibit epithelial proliferation, is around five times higher in concentration in human serum than in tear.[25] In our study, all of the eyes had remission to clear cornea after autologous serum treatment. The best corrected visual acuity also improved after the treatment of autologous serum. The increased corneal surface regularity and decreased epitheliopathy may have

contributed to the visual improvement. Schirmer's test failed to reach statistical significance after treatment due to a small number of cases. In summary, our results indicated the efficacy of autologous serum treatment for recalcitrant LINE patients. Autologous serum therapy was effective in the treatment of neurotrophic epitheliopathy compared with conventional treatment. None of our patients showed any ocular complications under careful preparation and storage of autologous serum eye drops.

In conclusion, risk factors of recalcitrant LINE in femtosecond laser-assisted myopic LASIK were identified as older age, a thinner flap (<110 μm), and a small flap diameter (<8.5 mm). Patients with recalcitrant LINE unresponsive to conventional treatment may benefit from autologous serum eye drops, which effectively improve corneal surface conditions and postoperative visual acuity.

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