Ascorbate Prophylaxis with Mitomycin-C for Corneal Haze after Laser-Assisted Sub-Epithelial Keratectomy

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ABSTRACT: **Background:** Corneal haze is a significant complication of photorefractive keratectomy (PRK) and laser-assisted sub-epithelial keratectomy (LASEK).

Objectives: To evaluate the effect of ascorbic acid supplementation in addition to perioperative topical mitomycin-C for the prevention of haze after LASEK.

Methods: We performed a retrospective, non-randomized case series study of two groups of 48 consecutive patients (96 myopic eyes) who had LASEK surgery. The treatment group was given ascorbic acid (vitamin C) orally, 500 mg, twice daily from 1 week before to 2 weeks after surgery. The control group was not offered any additional treatment. Ascorbate supplementation was the only difference in the postoperative treatment protocol between the treatment and control groups. Haze was assessed on a scale from 0 to 4 at the 1 year visit.

Results: Overall, 33.3% and 37.5% of the patients in the treatment and control groups respectively developed corneal haze. The trend of increased haze severity in the control group did not reach statistical significance.

Conclusions: Our results showed that systemic ascorbate supplementation does not have an additional effect on the prevention of haze after LASEK compared to the effect of topical mitomycin-C alone.

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KEY WORDS: laser-assisted sub-epithelial keratectomy (LASEK), mitomycin-C, ascorbic acid corneal haze, photorefractive keratectomy (PRK)

Corneal haze is a significant complication of photorefractive keratectomy and laser-assisted sub-epithelial keratectomy. Loss of corneal transparency after excimer laser photoablation is due to stromal changes induced by the wound-healing process. Haze occurs in different variants. Anterior stromal haze appears and peaks in severity 1–3 months after PRK and gradually declines over the following weeks. Late-onset corneal haze may develop 4–12 months after surgery, after haze-free interval, or without any previous opacity [1,2]. Late-onset corneal haze may lead to permanent scarring and is considered a serious complication. The frequency and severity of this complication in a large PRK series varies considerably and depends on the level of the myopic treatments [3,4]. Haze is considered a serious problem due to the reduction in visual outcome, the promotion of regression of the obtained refraction, and the possibility of creating glare. Therefore, prevention of haze after PRK or LASEK is important for achieving an optimal final surgical outcome.

Ascorbic acid is an important modulator of collagen production, acting as a cofactor for the hydroxylation of proline and lysine residues in procollagen. Hydroxyproline stabilizes the collagen triple-helix structure, a requirement for the secretion of procollagen and its processing of collagen. Ascorbic acid enhances the production of type I and type III collagen peptides in cultured rabbit keratocytes [5]. Ascorbate has also been shown to aid in the healing of alkali-burned corneas in rabbits, with a reduction in the loss of stromal glycosaminoglycans and increased deposition of basal lamina beneath new epithelia in the corneas treated with ascorbate or l-ascorbic acid 2-phosphate, as compared to controls [6].

An elevated concentration of ascorbate (1.1 mM) is found in the aqueous humor, which represents a 25-fold increase over the serum concentration [7]. This concentration results from active transport from plasma into the posterior chamber. The reason for this particular increase is unknown; however, its effect may relate to its ability to protect the eye from ultraviolet light damage and thus reduce free radical formation [8,9].

Research has shown that experimental elevations in ascorbic acid levels in animals had a protective effect against ultraviolet light-induced DNA damage to lens epithelium [10]. Stojanovic and Nitter [11] reported seasonal fluctuations in late-onset corneal haze prevalence after PRK, noting that these fluctuations co-varied with changes in environmental UV radiation at a particular latitude. Environments with high UV radiation levels might increase the risk for late-onset cor-

*The first two authors contributed equally to this study

PRK = photorefractive keratectomy

LASEK = laser-assisted sub-epithelial keratectomy
UV = ultraviolet

neal haze after PRK for moderate to high myopia. This was the rationale for the recommendation of saturation of the anterior eye with systemic ascorbate prior to excimer laser surgery in order to reduce postoperative haze and regression [12].

The therapeutic approach with systemic ascorbic acid corneal haze prophylaxis was tested in a retrospective non-randomized clinical study that suggested that oral ascorbic acid supplementation may have a prophylactic effect against haze development. Randomized prospective clinical trials were recommended for further investigation of its efficacy [13]. In the present study we retrospectively examined possible additional effects of ascorbic acid oral supplementation on corneal haze after LASEK surgery where mytomicin-C was used as the primary haze prophylaxis.

PATIENTS AND METHODS

Myopic eyes with or without astigmatism of 56 consecutive patients were treated with LASEK between October 2005 and December 2007 and followed for 12 months. All patients were treated according to the same protocol. The control group (without ascorbate) comprised 58 eyes of 29 patients operated between October 2005 and December 2006, and the therapeutic group (with ascorbate supplementation) comprised 54 eyes of 27 patients operated between January 2007 and December 2007.

Exclusion criteria were age (under 18 years old), chronic eye disease (cataract, glaucoma, uveitis, keratoconus, dry-eye syndrome) and systemic disorders (connective tissue disease, diabetes mellitus). Inclusion criteria were minimum follow-up time of 12 months and local corticosteroid treatment discontinuation within 3 months of surgery.

The preoperative evaluation included general and eye medical histories, uncorrected visual acuity, manifest and cycloplegic refractions, best corrected visual acuity, slit-lamp examination, keratometry, corneal topography, central corneal pachymetry, intraocular pressure, tear-film function assessment, scotopic pupillometry, and dilated fundus examination. Daily-wear soft contact lenses were removed 1 week before examination, and gas-permeable hard contact lenses 3–4 weeks before examination.

Each patient was informed about laser refractive surgery in general and LASEK in particular. All patients were required to read and sign a LASEK consent form as well as an informed consent form for the ascorbic acid oral supplementation.

Preoperative medications consisted of diazepam (Valium®, Roche, Switzerland) 10 mg per os, and ciprofloxacin 0.3% (Ciloxan®, Alcon, UK) and proparacaine 0.5% (Localin®, Fisher Pharmaceutical Labs, Israel) drops three times every 5 minutes. On the operating table, the eyelids were prepared with povidone-iodine 10% (Betadine®, Mundipharma, Switzerland); surgical drape was used to isolate the lashes, and a lid speculum was inserted.

Under the laser operating microscope, a guarded 8.5 mm 270-degree blunt trephine was centered on the pupil, pressed downward and rotated approximately 5 to 10 degrees to cut through the corneal epithelium, leaving a hinge at 12 o'clock. Subsequently, an 8.5 mm retaining well was centered on the trephine mark and filled with a 20% alcohol solution. After 30 seconds the alcohol solution was absorbed with a sponge. The eye surface was then rinsed with several drops of balanced salt solution (BSS®, B. Braun Medical Inc., USA).

The peripheral cornea was dried with a sponge to reveal the trephine mark. The epithelial flap was elevated and gathered at its 12 o'clock hinge. Myopia or myopic astigmatism was treated with the Allegretto 400 Hz excimer laser. The optical zone ranged from 5.5 to 7 mm, with a transition zone of 1.0–1.5 mm, amounting to a total ablation diameter of 6.5–8.5 mm. After laser ablation, a soaked pledget of mitomycin C 0.02% (Mitomycin-C 'Kyowa'®, Kyowa, UK) was placed on the ablated surface for 15 seconds if the preoperative myopic spherical equivalent ranged from -2.00 to -4.00 D, and for 30 seconds if it ranged from -4.01 to -10.00 D. The cornea was then irrigated with chilled BSS and the epithelial flap was returned to its approximate original position. A bandage soft contact lens was applied, and the drape and eyelid speculum were removed. Epithelial flap and contact lens position were examined under laser microscope. All patients were operated by the same surgeon.

The postoperative medications included fluorometholone 0.1% (FML®, Allergan, USA), ciprofloxacin 0.3% (Ciloxan) and artificial tears four times daily each. Patients were also given tetracaine 0.05% drops (one-tenth the anesthetic concentration) and acetaminophen tablets (Acamol®, Teva, Israel) to use as needed for pain. The contact lenses were removed on the fourth postoperative day in most of the patients. In ten patients contact lenses were left in place for an additional 1–2 days to allow for complete epithelial healing. FML drops were prescribed three to four times daily in the first postoperative month, twice daily in the second month, and once daily in the third month.

Regular follow-up examination was performed on day 1, day 5, the first month, the third month, and 12 months or earlier if needed after the surgery. Information at postoperative visits was collected, including patient symptoms and comments, medications, UCVA, manifest refraction, BCVA and slit-lamp examination. The haze was assessed at the 12 month visit according to a scale from 0 to 4, with 0 = clear cornea, 0.5 = trace opacity (barely discernable at the slit-lamp), 1 = mild, easily seen but not affecting refraction and vision, 2 = moderate, dense patches with difficult refraction affecting the vision, 3 = dense haze partially obscuring iris details and preventing

BSS = balanced salt solution
UCVA = uncorrected visual acuity
BCVA = best corrected visual acuity

refraction, and 4 = dense haze completely obscuring iris details.

Patients' data were analyzed using the SigmaStat 3.0 program, with each eye considered separately. Mann-Whitney rank sum test was used to assess statistical significance.

Ascorbate supplementation was the only difference in treatment protocol between the treatment and control groups. Treated patients were given ascorbic acid (vitamin C) orally at a dose of 500 mg twice daily starting 1 week before to 2 weeks after surgery. Patients in the control group did not receive any other vitamin supplementation or any other medication concurrently. Compliance was monitored by specifically asking the patients at follow-up examinations about their ascorbate intake.

RESULTS

The present study comprised 48 participants (96 eyes) – 24 in the treatment group and 24 in the control group. Eight patients did not appear at the 12 month visit (three of them were given ascorbate). The preoperative myopic spherical equivalent ranged from -2.00 to -10.00 diopters: 28 eyes (29.2%) -2.00 to -4.00 D, 44 eyes (45.8%) -4.01 to -8.00 D, and 24 eyes (25%) -8.01 to -10.00 D. The preoperative astigmatism ranged from 0 to +3.00 D. The patients' age ranged from 18 to 43 years; 43.8% of the patients were men and 56.2% were women.

Age, gender distribution and spherical equivalent refraction for both groups were similar [Tables 1 and 2]. The haze levels are given in Table 3. We found an association of haze occurrence and level with the preoperative refractive error [Table 4], with more haze in -4.01 t o -8.00 D and -8.01 to -10.00 D myopic groups. There were two cases of severe haze (grade 3), one at the 8 month visit in the study group with ascorbate supplementation (case 1) and the other (case 2) at the 10 month visit in the control group without ascorbate supplementation. The manifest refraction in case 1 before the treatment was -8.25–0.50×100 and BCVA was 20/20. At the 8 month visit there was grade 3 haze, manifest refraction -2.25–1.00×115, UCVA 20/200 and BCVA 20/30. The patient was immediately placed on topical Pred Forte 1% (Allergan, USA) eight times a day. During the next 6 months the examination showed trace haze, manifest refraction -0.75–0.50×95, BCVA 20/20 and UCVA 20/30. The manifest refraction in case 2 before the treatment was -9.00–1.25×180 and BCVA was 20/25. At the 10 month visit there was grade 3 haze, manifest refraction -1.50, UCVA 20/50 and BCVA 20/30. The patient was placed on topical Pred Forte 1% (Allergan, Inc) eight times a day. During the next 7 months the examination showed trace haze, manifest refraction -0.50–0.25×160, BCVA 20/20 and UCVA 20/25. Both case 1 and case 2 reported normal compliance in the period after the operation and the quality of the LASEK flaps was good.

D = diopter

Table 1. Age and gender distribution in groups with and without ascorbate supplementation

	LASEK with ascorbate supplementation	LASEK without ascorbate supplementation
Males	11	10
Females	13	14
Mean age ± SD (yrs) (range)	24 ± 5.8 (18–43)	27 ± 4.3 (19–43)

Table 2. Myopia ranges of treated eyes with and without ascorbate supplementation

Myopia range	LASEK with ascorbate supplementation	LASEK without ascorbate supplementation
-2.00 to -4.00 D	13 eyes (27%)	15 eyes (31%)
-4.01 to -8.00 D	23 eyes (48%)	21 eyes (44%)
-8.01 to -10.00 D	12 eyes (25%)	12 eyes (25%)

Table 3. Corneal haze levels at 12 month visit after LASEK

Grade of haze	LASEK with ascorbate supplementation	LASEK without ascorbate supplementation
0	32 eyes (66.7%)	30 eyes (62.5%)
1	10 eyes (20.8%)	11 eyes (22.9%)
2	5 eyes (10.4%)	6 eyes (12.5%)
3	1 eye (2.08%)	1 eye (2.08%)
Overall no. of eyes with haze	16 eyes (33.3%)	18 eyes (37.5%)

*$P = 0.719$

Table 4. Distribution of 34 eyes with corneal haze 12 months after LASEK considering haze severity and preoperative refraction

Corneal haze severity	-2.00 to -4.00 D (No. of eyes)	-4.01 to -8.00 D (No. of eyes)	-8.01 to -10.00 D (No. of eyes)
1	4	10	7
2	2	4	5
3			2
Sum (% of group)	6 (21.4%)	14 (31.8%)	14 (58.3%)

No statistically significant difference in haze prevalence or haze severity distribution was found between the treatment and control groups ($P = 0.719$). An insignificant mild trend for higher incidence and more severe haze was observed in the control group compared to the ascorbate-treated study group.

DISCUSSION

One possible unwanted effect of LASEK surgery is corneal haze. Corneal haze development can lead to myopic regres-

sion and BCVA reduction. The healing processes in the cornea are likely to play a significant role in haze formation [14].

The reason why some patients are more prone to haze after refractive surgery than others is poorly understood. To some extent the explanation may be found in environmental factors. Haze after PRK was found to be unfavorably influenced by UV radiation in an experimental setting [15].

Acute corneal changes following excimer laser surgery in rabbits are favorably modified by ascorbate treatment [16]. This observation refers to application of a topical ascorbate solution confined to a time span of 24 hours after surgery when the animals were killed and processed for morphological studies. Oxygen radical tissue damage, quantified in terms of decreased number of lipid peroxidation compounds and polymorphonuclear cells, was shown to be reduced in the treated eyes. Ascorbate reservoirs of the anterior eye, including corneal epithelium, corneal stroma and aqueous humor, are filled from serum provided through an adequate systemic supply [13].

In the present study 33.3% and 37.5% of patients in the treated and control group, respectively, developed corneal haze [Table 3]. A minimal trend in favor of the ascorbate treatment group in the haze levels at the 12 month visit was statistically insignificant ($P = 0.719$). No adverse effects of systemic application of ascorbate were noted.

Compliance was monitored by specifically asking the patients at follow-up examinations about their ascorbate intake. Measuring vitamin C levels in blood is possible as compliance monitoring; however, since it is complicated and invasive it was not used in the current study. The tablets were dispensed by our team. We recorded both used and remaining tablets.

The protocol followed in both groups in our study included the use of intraoperative mitomycin C and postoperative local corticosteroids due to their well-documented effect on haze prevention [17-19]. This limits the applicability of our conclusion regarding the efficacy of ascorbate. Theoretically we could have refrained from using these two agents in the intra and postoperative periods in order to demonstrate the true effect of ascorbate, but we considered this to be ethically unacceptable due to the proven beneficial effect of these agents on haze formation.

Our study demonstrated good quality flaps in 90% of the LASEK flaps. Furthermore, no correlation was found between inferior quality LASEK flaps and haze incidence.

CONCLUSIONS

Our results do not encourage ascorbate supplementation for prevention of haze after LASEK if perioperative mitomycin-C and postoperative local steroids are used.

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