Original Article

Subclinical inflammatory response after accelerated corneal cross-linking

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Background: To evaluate the inflammatory response after accelerated collagen cross-linking (CXL) in eyes with keratoconus.

Methods: Consecutive eyes with keratoconus undergoing CXL surgery were included in this non-randomized interventional study. Aqueous flare was measured pre- and post-operatively with a laser flare photometer at 1 week, 1, 3 and 6 months after CXL.

Results: Sixty eyes of 60 patients were entered into the study. Before CXL, the mean flare value was 4.5 photons per millisecond (ph/ms). The flare values observed at week 1 (7.1 ph/ms; P=0.008), month 1 (6.5 ph/ms; P=0.04), month 3 (6.7 ph/ms; P=0.004) and month 6 (6.7 ph/ms; P=0.004) were significantly higher compared to baseline. Flare values were not significantly different from week 1 up to 6 months after CXL (P=0.930). No statistically significant correlation was detected between the amount of inflammation and keratometric indices.

Conclusions: Accelerated CXL in patients with keratoconus may cause a subclinical inflammatory response which is evident as slight but rather long-lasting rise of aqueous flare.

Keywords: Keratoconus; accelerated cross-linking; clinical trial; inflammation

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Introduction

Corneal collagen cross-linking (CXL) is a recognized technique for slowing or halting the progression of keratoconus using riboflavin and UV light which results in corneal strengthening through the formation of covalent bonds in the corneal stroma. In the standard technique described by Wollensak *et al*., the riboflavin-treated eye is illuminated for 30 minutes by UVA 370 nm light at an irradiance of 3 mW/cm^2 (cumulative dose 5.4 J/cm^2) (1). Today, there is growing interest in the accelerated approach in which the procedure time is reduced by increasing the irradiation power and decreasing the exposure time (2-4). Prior studies have shown a significant short-term rise of aqueous flare following refractive photo ablative surgeries with or without lamellar keratectomy (5-8). In this study we aimed to evaluate flare as an indicator of the inflammatory response following accelerated CXL.

Methods

This prospective, non-randomized interventional before-after study was performed at Noor Eye Hospital, Tehran. All patients, enrolled from September 2012 to January

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2013, were informed, and consent was obtained after the procedure had been explained. The study protocol was approved by the Institutional Review Board of Noor Ophthalmology Research Center (IRB# M.1118).

Patients with mild to moderate keratoconus [maximum K less than 55 diopters (D)] and the following inclusion criteria were enrolled: (I) age between 18 and 35 years; (II) best-corrected visual acuity (BCVA) of 20/40 or worse; (III) topographic evidence of progressive keratoconus defined as ≥1.0 D increase in maximum keratometry and/or the manifest cylinder, or ≥0.5 D increase in refraction spherical equivalent over 24 months (3); (IV) minimum corneal thickness of 450 µm. Patients with a history of prior ocular surgeries, inflammatory ocular or systemic disease (e.g., diabetes) and recent contact lens usage were not enrolled.

Before CXL, all eyes underwent complete ophthalmic examinations including measurement of uncorrected visual acuity (UCVA) and BCVA, slit-lamp biomicroscopy and fundus examination, corneal Scheimpflug imaging (Pentacam, OCULUS, Inc., Lynnwood, WA, USA) and laser flare photometry (FM-600; Kowa, Tokyo, Japan). All examinations were repeated at 1 week, 1, 3, and 6 months after CXL.

Surgical technique

The eye was anesthetized by instillation of one drop of tetracaine hydrochloride 0.5% (Anestocaine 0.5%, Sina-Darou Pharm. Co., Tehran, Iran). After removal of the epithelium from the central 7 mm of the cornea, 0.1 mL of riboflavin 0.1% in dextran solution 20% (Streulipharmeceuticals, Uznach, Switzerland) was instilled onto the cornea every 3 minutes for a total time of 30 minutes. Accelerated CXL was performed using 5 minutes of continuous UVA 370 nm light (IROC UVX system, Zürich, Switzerland) at an irradiance of 18 mW/cm^2 (cumulative dose 5.4 J/cm^2). A therapeutic soft contact lens (Night & Day, Ciba Vision, Duluth, GA, USA) was fitted at the end of the procedure. All patients were treated post-operatively with levofloxacin 0.5% and betamethasone 0.1% 4 times daily for 1 week and were examined daily until complete healing of the epithelium when the bandage contact lenses were removed.

Anterior chamber flare measurement

Anterior chamber flare was measured with a regularly calibrated laser flare photometer (FM-600, Kowa, Tokyo, Japan). For each eye, seven consecutive readings were obtained from the lower third of the anterior chamber with <10% background scatter. The two extreme readings were crossed out, and the average of the remaining five was recorded as the flare value in photons per millisecond (ph/ms).

Statistical analysis

Statistical analysis was performed using the repeated measures analysis of variance to compared results of the pre- and postoperative examinations. For the analysis of associations between quantitative variables, the Pearson correlation test was applied. A P value <0.05 was considered statistically significant.

Results

Sixty eyes of 60 patients with mild to moderate keratoconus were entered into this study. Mean age of the patients was 23.8 years (range, 15–35 years). Before CXL, their mean UCVA was 0.71±0.51 logMAR and improved to 0.52±0.42 logMAR 6 months after surgery (P=0.039). Mean BCVA was also improved from 0.17±0.28 logMAR preoperatively to 0.11±0.14 logMAR 6 months after surgery (P=0.011). *Table 1* summarizes clinical profiles before CXL and 6 months after the procedure.

The mean anterior chamber flare increased from 4.5 ph/ms before CXL to 7.1±7.5 ph/ms 1 week post-operatively (P=0.008). Although the mean flare value decreased slightly to 6.5±4.8 ph/ms at 1 month, it was still significantly higher than baseline (P=0.040). The changes were rather steady from month 1 up to the last visit (*Figure 1*). There were no statistically significant differences between mean flare values at week 1 and other follow-up visits.

Discussion

The postoperative inflammatory response remains an important determinant of corneal wound healing. Laser flare meters allow for the quantification of the inflammatory response through objective measurement of the aqueous flare. With a high degree of accuracy and sensitivity, laser flare meters can detect minute alterations in the blood-aqueous-barrier function which may not be clinically detectable (9).

Previous studies have shown the induction of an inflammatory response following photo ablative refractive surgeries. Tomas-Barberan and Fagerholm described

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Table 1 Patients' clinical profiles at baseline and 6 months after accelerated corneal collagen cross linking

Parameters	Before CXL	After 6 months	P value
UCVA (Log MAR)	0.70±0.51	0.52±0.42	0.005
BCVA (Log MAR)	0.17±0.28	0.11±0.14	0.084
Average mean-K (D)	45.45±2.01	45.42±3.07	0.362
Maximum keratometry (D)	48.32±3.57	48.02±3.54	0.116
Central corneal thickness	478.96±65.73	470.82±66.05	0.006

UCVA, uncorrected visual acuity; BCVA, best-corrected visual acuity; CXL, collagen cross-linking.

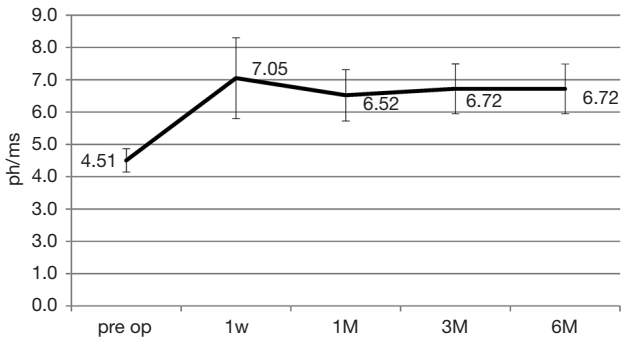

Figure 1 Mean anterior chamber flare with corresponding error bar depicting 95% confidence interval before, 1 week, 1, 3 and 6 months after CXL. ph/ms, photons per millisecond; CXL, collagen cross-linking.

a significant increase in anterior chamber flare after photorefractive keratectomy (PRK) (5). Others reported a short-lasting subclinical inflammation following uneventful laser *in situ* keratomileusis in virgin eyes (6) as well as corneal grafting (7). Pisella *et al.* showed that photo ablative refractive surgery with or without lamellar keratectomy could induce alteration in flare which is correlated with the depth of ablation (8).

In our study, following the accelerated CXL with UVA light at an irradiance of 18 mW/cm^2, an early and rather steady increase of aqueous flare occurred. Contrary to previous reports, the rise of aqueous flare was not short-term and flare intensity did not return to baseline even up to 6 months after CXL. We speculate that the production of inflammatory mediators may have a causative role for this observation. During CXL, riboflavin molecules absorb the UVA light. In the presence of oxygen, a photo-oxidative reaction occurs and results in the excitation of riboflavin into a triplet state and generation of singlet oxygen. Excited riboflavin and oxygen free radicals catalyze biochemical reactions which result in the formation of additional covalent bonds between collagen fibers. The biochemical reaction and release of free radicals, as well as apoptosis of keratocytes lead to the local production of inflammatory cytokines which will subsequently affect the blood-aqueous-barrier function. Prior studies have shown that both mechanical and photochemical keratectomy produce inflammatory mediators such as PGE2, an observation that supports a role for cyclo-oxygenase inhibitors in postoperative therapy (10,11).

The rise of aqueous flare following CXL may be attributed in part to the surgical trauma to corneal tissue secondary to epithelial removal. However, the same surgical trauma during PRK only causes a temporary rise of aqueous flare (5,8). Therefore, the rather long-term rise of flare could not be attributed to the surgical trauma and hence, the causative role of UVA irradiance in disrupting the blood-aqueous barrier function is a more likely explanation. Another possible explanation for the increased aqueous haze would be the haze induced by CXL. Greenstein *et al.* (12) demonstrated that haze reaches a maximum at the first month after CXL, remains relatively stable until the third month, and takes a downward trend afterwards to the 12th month. The postoperative formation of new covalent bonds and corneal stromal changes can be another reason for this subclinical inflammation. However, the inflammation didn't cause the clinical complications and VA was significantly improved at this time as well. Several studies have demonstrated the safety and efficacy of CXL with the standard (13,14) and accelerated (2,15-17) approaches. There is no similar study on cases receiving standard CXL, therefore, it is not clear whether lower irradiance of UVA in standard CXL procedure will induct such an inflammatory response or not. A limitation of this stay was the short follow-up time. Also, studying inflammatory changes in a non-operated group of keratoconus patients would allow for a more accurate conclusion about CXL-related changes in flare. Further studies and longer follow-ups are needed.

Conclusions

In conclusion, the employment of higher fluence CXL with shorter exposure time has recently been proposed as an alternative to standard CXL technique in stabilizing

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keratoconus. Our study shows that the procedure may cause a subclinical inflammatory response which is evident as slight but a rather long-lasting rise of aqueous flare.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by Institutional Review Board of Noor Ophthalmology Research Center (IRB# M.1118) and written informed consent was obtained from all patients.

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