Epithelium-Off Corneal Collagen Cross-linking Versus Transepithelial Cross-linking for Pediatric Keratoconus

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Purpose: To compare efficiency and safety of epithelium-off corneal cross-linking (CXL) and transepithelial cross-linking (TE-CXL) in pediatric patients with progressive keratoconus.

Methods: Uncorrected and corrected visual acuity, corneal topography and pachymetry (Pentacam; Oculus Pentacam), and in vivo confocal microscopy (HRT II, Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany) were evaluated at baseline and at 3, 6, and 12 months.

Results: In the epithelium-off CXL group (19 patients, 23 eyes; mean age, 14.75 ± 2.1 years), a significant improvement at month 12 was present for Kmax [−1.11 diopters (D), $P = 0.01$], Kmin (−3.2 D, $P = 0.001$), mean K (−1.47 D, $P = 0.01$), surface asymmetry index (−0.64 D, $P = 0.001$), inferior–superior symmetry index (−0.54 D, $P = 0.01$), index of height asymmetry (−2.97, $P = 0.03$), and anterior elevation at the thinnest location (−2.82 D, $P = 0.01$) and at the apex (−2.27 D, $P = 0.01$). Postoperative corneal edema lasted 3 months in 16 eyes (69.5%) and more than 6 months in 2 eyes (8.7%). In the TE-CXL group (10 patients, 14 eyes; mean age, 15 ± 4.2 years), a significant improvement at month 12 was present for Kmax (−1.14 D, $P = 0.02$), Kmin (−2.04 D, $P = 0.01$), mean K (−1.63 D, $P = 0.01$), surface asymmetry index (−0.86 D, $P = 0.001$), inferior–superior symmetry index (−0.55 D, $P = 0.001$), index of height asymmetry (−2.95, $P = 0.01$), and anterior elevation at the thinnest location (−2.96 D, $P = 0.01$) and at the apex (−2.19 D, $P = 0.01$). No postoperative corneal edema after TE-CXL was observed. Changes at month 12 from baseline were not significantly different between the 2 groups ($P > 0.05$). TE-CXE was significantly less painful than epithelium-off CXL.

Conclusions: In pediatric patients with progressive keratoconus, TE-CXL was less painful, provided similar effectiveness and fewer complications than epithelium-off CXL at 12-month follow-up.

Key Words: pain, keratoconus, cross-linking, children, Pentacam

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Keratoconus is a noninflammatory process in which the corneal architecture deforms in association with biomechanical weakening. Pediatric age at the time of diagnosis represents a negative prognostic factor for keratoconus progression, with high risk for more rapid progression of the disease and increased probability of corneal transplant.[1] Riboflavin UVA corneal collagen cross-linking (CXL) consists of irradiation with UVA (360 nm wavelength) after application of 0.1% riboflavin in de-epithelialized corneas and represents the only approach to progressive keratoconus to delay its progression and to reduce or eliminate the need for donor keratoplasty.[2–4] CXL initiates photopolymerization of the corneal collagen, causing additional covalent bonds within collagen fibrils and the quaternary structure of the collagen and consequent corneal stiffening while maintaining structural integrity and corneal transparency and preserving corneal and ocular anatomical and histological structures. Long-term clinical studies have shown that CXL with epithelium debridement (traditional CXL, epithelium-off CXL) slows and in most cases blocks progression of keratoconus,[5–8] and in some cases, causes an improvement of refractive and topographic features.[3,9,10] CXL performed by administering 0.1% riboflavin−20% dextran solution without epithelial debridement (transepithelial cross-linking, TE-CXL) has shown an efficacy reported to be similar to that of the epithelium-off CXL.[3,11] To date, no reports on utilization of TE-CXL in pediatric patients are known. Herein, we compared TE-CXL and epithelium-off CXL for treatment of progressive keratoconus in pediatric patients.

METHODS

The study was performed in accordance with the ethical standards described in the 2000 revision of the 1964 Declaration of Helsinki. Institutional Review Board approval was obtained from the University of Naples Federico II for this study. We retrospectively reviewed the data of all patients younger than 18 years (range, 12–18 years) and with a diagnosis of keratoconus who underwent epithelium-off CXL and TE-CXL at the University Federico II and Pellegrini Hospital, Naples, and at Niguarda Hospital, Milan. Inclusion criteria were age younger than 18 years, confirmed keratoconus,

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The study was performed with informed consent and following all the guidelines for experimental investigations required by the Institutional Review Board or Ethics Committee of which all authors are affiliated.
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central corneal thickness greater than 400 μm, disease progression documented by an increase in the maximum cone apex curvature of at least 1.00 diopter (D), an increase in central corneal astigmatism of at least 1.00 D over the previous 6 months, clear cornea at biomicroscopic examination, absence of reticular dark striae at confocal laser microscopy in vivo, and subjective loss of vision. Dark striae (also named Vogt striae) are observed in the posterior stroma and Descemet layer in the keratoconic cornea and represent collagen lamellae under stress.[12,13] Patients were examined after 6 months if the progressive pattern was not obvious or was confused by rigid contact lens wear. Both parents of each patient signed an informed consent form. Contact lens wearers were instructed to discontinue spherical soft lenses for a minimum of 4 weeks and soft toric, rigid gas-permeable and hard lenses for a minimum of 12 weeks before the preoperative eye examination. Exclusion criteria were central corneal opacities, a history of herpetic keratitis, severe dry eye, concurrent corneal infections, concomitant autoimmune diseases or any ocular surgery, abnormality in lens or retina on biomicroscopic examination, and patients too young or not cooperative enough to perform CXL.

Consecutive patients considered eligible for treatment between May 2006 and September 2009 underwent epithelium-off CXL, whereas consecutive patients included between October 2009 and January 2011 underwent TE-CXL.

Epithelium-Off Collagen Cross-linking

Epithelium-off CXL was performed according to the methodology described by Wollensak et al[2] Topical anesthesia was administered and the corneal epithelium removed by mechanical debridement over the central 9.0 mm. To reduce the risk for UV exposure of retroirideal eye structures, miosis was induced with 1.0% pilocarpine 30 minutes before the procedure. Riboflavin (0.1% in 20% dextran solution; Ricrolin; Sooft, Montegiorgio, Italy) was then administered topically every 2 minutes for 30 minutes. The cornea was exposed to UVA 365 nm light (Vega CBM X linker; Costruzione Strumenti Oftalmici, Florence, Italy) for 30 minutes at an irradiance of 3.0 mW/cm^2. During UVA exposure, riboflavin drops were continued every 2 minutes. Postoperatively, antibiotic and corticosteroid drops were administered and a therapeutic soft contact lens (Acuvue) was placed. The contact lens was removed after epithelial healing, typically 3 to 5 days postoperatively. Antibiotic drops were continued for 1 week, and corticosteroid drops were continued for 2 weeks.

TRANSEPITHELIAL CROSS-LINKING

To perform TE-CXL, corneal imbibition was obtained with 0.1% riboflavin–15% dextran solution supplemented with Tris–hydroxymethyl aminomethane and sodium EDTA (Ricrolin TE; Sooft) by instillation of 2 drops every 5 minutes for 30 minutes. One drop of 1% pilocarpine was administered 30 minutes before treatment. Twenty minutes before UV radiation, the cornea was anesthetized with single-dose anesthetic eye drops (4% lidocaine), 1 drop every 4 minutes. The periocular skin was disinfected for 5 minutes with povidone–iodine diluted to 10.0%. A blepharostat was applied, and a ring-shaped silicone container was used to improve riboflavin penetration. Ricrolin TE was administered every 5 minutes throughout the treatment, and slit-lamp examination was performed to confirm that sufficient riboflavin was in the stroma. Corneal irradiation with an UVA source (Vega; Costruzione Strumenti Oftalmici) was then performed at 3 mW/cm^2 for 30 minutes. At the end of treatment, the eye was treated with ofloxacin antibiotic eye drops, and a therapeutic corneal lens bandage (Acuvue) was applied for 3 days. After therapeutic lens removal, antibiotic drops were continued for 1 week.

Preoperative and postoperative examination included uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), corneal topography and pachymetry (Pentacam; Oculus Pentacam), and in vivo confocal microscopy (HRT II, Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany). Visual acuity was evaluated using a LogMAR Early Treatment Diabetic Retinopathy Study chart at 4 m. Glare disability was tested with the brightness acuity tester (BAT; Marco Ophthalmic, Inc).[14,15] Monocular logarithm of the minimum angle of resolution acuities (high and low contrast) and contrast sensitivities (measured with Pelli–Robson contrast sensitivity chart) were evaluated in the presence and absence of BAT glare source at the brightest setting (1370 candela/m^2). Patients were followed up at 3-month intervals during 12 months. Results at baseline, month 3, month 6, and month 12 are given. Complications of treatment, including corneal edema, haze (stromal hyperdensity), re-epithelization delay (in epithelium-off CXL group), hyperemia, and photophobia, were recorded.

Subjective postoperative pain evaluation was evaluated by the patients using the Wong–Baker FACES Pain Rating Scale[16]—a visual analog scale—and recorded in a standard table. In this self-report structured questionnaire, the patients are asked to choose the face that best described the degree of their pain, ranging from 0 to 5 (0 = no pain; 1 = discomfort; 2 = light pain; 3 = moderate pain; 4 = intense pain; 5 = unbearable pain). The pain level should correspond to the pain felt during the respective day, not only at the moment of evaluation.

Statistical analyses were performed with a commercially available software package (SPSS version 13.0; SPSS, Inc, Chicago, IL). All data are reported as mean (SD). Repeated-measures analysis of variance was used to test intragroup variations of each evaluated parameter from baseline to month 3, month 6, and month 12. Paired t test was used to check the intergroup difference for change from baseline of evaluated parameters at each follow-up visit. The level of statistical significance was set at $P < 0.05$.

RESULTS

Thirty patients (39 eyes) were included in the study. Mean age was 15.2 ± 1.7 (12–17) years with an overall male to female ratio of 3:1. Characteristics of patients at baseline and during 12 months of follow-up are shown in Table 1.

In the epithelium-off CXL group, 19 patients (14 adolescent boys, 5 adolescent girls; 23 eyes) were included, with a mean age of 14.75 ± 2.1 years. Eight patients (42.1%)

TABLE 1. Visual Acuity, Topographic and Confocal Microscopy Data Before and After Epithelium-Off CXL and TE-CXL in Pediatric Patients

	Epithelium-Off CXL, n = 23 Eyes, 14 M/5 F				
	BL	**Month 3**	**Month 6**	**Month 12**	***P***
UCVA, logMAR	0.68 ± 0.21	0.54 ± 0.21	0.66 ± 0.31	0.67 ± 0.24	0.1
BCVA, logMAR	0.36 ± 0.1	0.31 ± 0.2	0.33 ± 0.2	0.36 ± 0.1	0.8
Kmin, D	46.31 ± 3.4	42.32 ± 4.2*,†	43.11 ± 3.6*,†	43.13 ± 3.5*	0.001
Kmax, D	50.13 ± 4.0	48.83 ± 4.0*,†	49.38 ± 3.6*,†	49.02 ± 4.6*	0.01
Mean K, D	47.11 ± 3.5	46.81 ± 3.3	46.31 ± 3.7	45.64 ± 4.3*	0.01
Astigmatism, D	5.26 ± 3.4	5.05 ± 3.2*,†	5.08 ± 2.9*,†	5.21 ± 3.3	0.3
CCT, μm	487.2 ± 1 5.1	509 ± 18.2	491 ± 20.1	492 ± 22.1	0.5
Endothelial cell count, cells/mm^2	3212 ± 331.1	3195 ± 295.2	3193 ± 221.3	3188 ± 147.1	0.7
Surface asymmetry index	6.76 ± 1.32	6.61 ± 1.22	6.39 ± 1.41*	6.12 ± 1.15*	0.001
Inferior–superior symmetry index, mm^2	7.40 ± 0.7	7.25 ± 0.7	7.21 ± 1.2	6.84 ± 1.1*	0.01
Anterior elevation, thinnest location, D	30.21 ± 15.11	26.12 ± 16.22†	25.32 ± 15.39†	27.39 ± 15.56*	0.01
Anterior elevation, apex, D	20.51 ± 16.39	17.39 ± 18.21†	16.46 ± 17.21†	18.24 ± 15.21*	0.01
Posterior elevation at the thinnest location, D	54.11 ± 28.74	52.18 ± 25.76	52.36 ± 27.65	52.10 ± 29.39	0.07
Posterior elevation at the apex, D	30.10 ± 22.11	30.12 ± 25.32	30.15 ± 23.29	30.8 ± 22.65	0.4
IHA	30.11 ± 15.8	31.21 ± 11.2	31.25 ± 14.3	27.14 ± 14.8*	0.03
	TE-CXL, n = 16 Eyes, 8 M/3 F				
	BL	**Month 3**	**Month 6**	**Month 12**	***P***
UCVA, logMAR	0.55 ± 0.33	0.46 ± 0.32	0.48 ± 0.21	0.54 ± 0.22	0.3
BCVA, logMAR	0.26 ± 0.2	0.15 ± 0.1	0.22 ± 0.4	0.27 ± 0.2	0.5
Kmin, D	45.26 ± 2.7	47.12 ± 5.3†	47.21 ± 3.1†	43.22 ± 3.1*	0.01
Kmax, D	49.27 ± 4.1	49.21 ± 5.0†	49.10 ± 5.1†	48.13 ± 5.4*	0.02
Mean K, D	47.50 ± 4.2	48.65 ± 4.1	48.66 ± 4.3	45.87 ± 4.0*	0.01
Astigmatism, D	5.22 ± 4.2	5.14 ± 4.4†	5.16 ± 5.3†	5.33 ± 5.1	0.5
CCT, μm	490.2 ± 22.3	490.1 ± 22.3	485.1 ± 17.1	488.0 ± 19.3	0.6
Endothelial cell count, cells/mm^2	3241.37 ± 330.1	3210.2 ± 2 21.12	3131 ± 127.2	3168.23 ± 223.9	0.5
Surface asymmetry index	7.18 ± 1.3	7.03 ± 1.2	7.01 ± 1.1	6.32 ± 1.4*	0.001
Inferior–superior symmetry index, mm^2	7.24 ± 0.4	7.18 ± 0.1	7.16 ± 0.2	6.69 ± 0.1*	0.001
Anterior elevation, thinnest location, D	29.27 ± 15.26	29.26 ± 17.31†	29.29 ± 16.28†	26.31 ± 15.32*	0.01
Anterior elevation, apex, D	19.68 ± 15.21	19.56 ± 20.11†	19.78 ± 15.01†	17.49 ± 21.08*	0.02
Posterior elevation at the thinnest location, D	53.36 ± 25.32	51.14 ± 26.39	51.28 ± 23.29	51.42 ± 26.87	0.07
Posterior elevation at the apex, D	29.59 ± 15.76	29.22 ± 26.12	29.27 ± 21.32	29.35 ± 19.39	0.5
IHA	29.34 ± 18.2	29.43 ± 12.5	29.54 ± 15.9	26.39 ± 12.1*	0.01

*Statistically significant change from baseline.
†Statistically significant difference between the 2 groups for change from baseline.
BL, baseline; CCT, corneal central thickness; F, females; IHA, index of height asymmetry; logMAR, logarithm of the minimum angle of resolution; M, males; *P*, statistical change at month 12 compared with baseline.

had worn lenses before treatment (soft lenses in 2 cases, rigid gas-permeable lenses in 6 cases). After treatment, UCVA, BCVA, corneal central thickness, and endothelial cell count remained stable at 12-month visit ($P > 0.05$), whereas a significant improvement at month 12 was present for Kmax (−1.11 D, $P = 0.01$), Kmin (−3.2 D, $P = 0.001$), mean K (−1.47 D, $P = 0.01$), surface asymmetry index (−0.64 D, $P = 0.001$), inferior–superior symmetry index (−0.54 D, $P = 0.01$), index of height asymmetry (−2.97, $P = 0.03$), and anterior elevation at the thinnest location (−2.82 D, $P = 0.01$) and at the apex (−2.27 D, $P = 0.01$). No significant difference was found in the posterior elevation at the thinnest location and at the apex from preoperative value at 1 year posttreatment. A significant improvement of Kmin, Kmax, and astigmatism was present at month 3 and month 6. Transient postoperative corneal edema with glare disability lasted 3 months in 16 eyes (69.5%) (in the absence of a statistically significant visual loss) and was well managed with topical preservative-free steroids (fluorometholone drops, tapered 3 times a day for 4–6 weeks). Only in 2 eyes (8.7%) corneal edema persisted until month 8 despite steroidal treatment. Haze did not occur, and no re-epithelization delay was noticed.

In the TE-CXL group 11 patients (8 males, 3 females, 16 eyes) were included, with a mean age 15 ± 2.1 years. Four patients (36.3%) had worn rigid gas-permeable contact lenses before treatment. After treatment, UCVA, BCVA, corneal central thickness, and endothelial cell count remained stable at 12-month visit ($P > 0.05$), whereas a significant improvement at month 12 was present for Kmax (−1.14 D, $P = 0.02$),

Kmin (−2.04 D, $P = 0.01$), mean K (−1.63 D, $P = 0.01$), surface asymmetry index (−0.86 D, $P = 0.001$), inferior–superior symmetry index (−0.55 D, $P = 0.001$), index of height asymmetry (−2.95, $P = 0.01$), and anterior elevation at the thinnest location (−2.96 D, $P = 0.01$) and at the apex (−2.19 D, $P = 0.01$). No significant difference was found in the posterior elevation at the thinnest location and at the apex from preoperative value at 1 year posttreatment. All corneas remained transparent on slit-lamp examination throughout the follow-up period, with no signs of haze or glare disability. Side effects of TE-CXL were transient hyperemia (16 of 16 eyes), foreign body sensation (11 of 16 eyes, 68.7%), and photophobia (16 of 16 eyes) during the first few hours and days after treatment.

A significant difference was present between the 2 groups as concerns change from baseline of Kmin, Kmax, astigmatism, anterior elevation at the thinnest location and at the apex, at month 3 and at month 6 ($P < 0.05$) (Table 1). At month 12, no significant differences as concerns change from baseline of each of the evaluated parameters were present between the 2 groups.

Mean postoperative pain was significantly higher in the epithelium-off CXL group than in the TE-CXL group in the immediate postoperative period on the same day as the surgery (2.9 ± 1.2 vs. 1.2 ± 1.1, respectively, $P = 0.01$) and in the first postoperative day (2.3 ± 1.2 vs. 0.9 ± 0.7, respectively, $P = 0.01$), whereas differences were not significant during following 4 days ($P > 0.05$). When bilateral CXL was performed, no difference in pain sensitivity was present between the 2 eyes in any postoperative evaluation in any group ($P > 0.05$). No difference in the pain sensitivity was present between sexes ($P > 0.05$).

DISCUSSION

To our knowledge, this is the first reported evaluation of TE-CXL in pediatric patients with progressive keratoconus. TE-CXL was compared with epithelium-off CXL. In TE-CXL, the enhancers trometamol and ethylenediaminetetraacetic acid are added to help riboflavin penetrate the corneal stroma through an intact epithelium, thereby avoiding the need for epithelial debridement. According to our data, both procedures seemed to halt the progression of keratoconus in all treated eyes over the 12-month follow-up. According to epidemiology findings, an overall male to female ratio of 3:1 was found. These results slightly differ from the 2:1 value reported by Rabinowitz[17] and the 4:1 value recently reported by Caporossi et al.[8]

In both groups, UCVA and BCVA improved although not significantly at month 3 and month 6 while decreased to baseline values at month 12. Pachymetry and endothelial cells count tended to remain stable during 12 months of follow-up while a similar significant improvement of topographic indices was present in both groups at month 12, reflecting improved corneal symmetry because of recentering of the corneal apex, as suggested in previous studies.[18] Combined action of 0.1% riboflavin (photosensitizing agent) and UVA irradiation induces release of singlet oxygen that photopolymerizes stromal collagen, reduces the lytic effect of collagenase, and increases corneal resistance to deformation, counteracting certain major pathophysiologic mechanisms of keratoconus.[2,19–21] Although TE-CXL in pediatric patients has never been reported, a significant and fast functional improvement in younger patients after Riboflavin UVA epithelium-off CXL was shown on a large cohort of patients with a long-term follow-up,[11] and several studies suggest epithelium-off CXL to represent the primary choice in young patient with progressive keratoconus.[6,7] Only one previous report showed a worsening trend or at least instability of keratoconus in a minority of patients (approximately 5%) younger than 18 years who had undergone traditional CXL.[8]

In our series, TE-CXL was shown to be safer than epithelium-off CXL. In the group treated with epithelium-off CXL, transient corneal edema with glare disability in the first postoperative 12 weeks was present in 80% of cases (without a statistically significant visual loss) and was managed with topical steroids. Only in 2 cases corneal edema persisted until month 8 despite steroidal treatment while haze did not occur. No re-epithelization delay was noticed. In the TE-CXL group, corneal edema and glare disability were never observed. Corneal edema is more frequent after epithelium-off CXL than after TE-CXL, presumably because of a greater exposure of corneal endothelium to UV damage after removal of epithelium.[3,11,13] Glare disability is caused by corneal edema and was found only in the epithelium-off CXL group. None of our patients had a postoperative infection. However, this complication after epithelium-off CXL has been described.[22] Formation of temporary corneal haze, corneal melting, permanent scars, endothelial damage, treatment failure, sterile infiltrates, and herpes reactivation are the other reported complications of epithelium-off CXL.[23–26] Sparing of corneal epithelium in TE-CXL is likely to provide greater safety than epithelium-off CXL.

In this study, mean postoperative pain was significantly higher in the epithelium-off CXL group than in the TE-CXL group on the day of the surgery and on the first postoperative day. No significant differences were noted during the following 4 days. Postoperative pain after epithelium-off CXL is because of the exposure of corneal nerves and release of inflammatory mediators, especially prostaglandins and neuropeptides. Postoperative pain after epithelium-off CXL has been shown to be intense, especially in the first 3 days, even with an aggressive pain control regimen.[27] TE-CXL allows respecting corneal nerve fibers, is devoid of endothelial toxicity, and is much less painful than traditional CXL.[28,29] Interestingly, Ghanem et al[27] showed that postoperative pain after epithelium-off CXL seems inversely related to patient's age. Our results agree with the degree of postoperative pain rating scale observed by Ghanem et al[27] in patients younger than 20 years. Murphy et al[30] showed an inverse correlation between corneal sensitivity and age in both nondiabetic and diabetic subjects, along with an increasing variability in the measured threshold. They suggested that the number of functional nerves in the peripheral sensory nervous system decreases with age, and those remaining become less efficient at transmitting signals to the central nervous system.

Limitations of the present study are the relatively small number of treated patients and the follow-up limited to 1 year.

Keratoconus is a progressive disease that can progress during several decades. Therefore, a longer follow-up is necessary to definitely assess the need for retreatment, the effect on trophicity, and the biomechanics of both procedures.

In conclusion, TE-CXL provided equivalent effectiveness and fewer complications than epithelium-off CXL at 1-year follow-up in pediatric patients with progressive keratoconus. TE-CXL was shown to be a less painful technique in the first postoperative days. A prospective long-term study on a larger cohort is planned to assess whether TE-CXL could represent primary choice for treatment in these cases.

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