Review

Updates on corneal collagen cross-linking: Indications, techniques and clinical outcomes

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Abstract

Purpose: To review the historical background and basic principles of collagen cross-linking, to bring together the data regarding the outcomes and complications of collagen cross-linking and finally to explore the efficacy and safety of new variations of this technique.
Methods: A literature review was performed using PubMed and Scopus. The following keywords were used for literature search: cross linking, crosslinking, cross-linking, keratoconus, keratectasia.
Results: In contrast to traditional treatment modalities for keratoconus (KCN), this new technique addresses the progression of the disease. Several clinical studies have been conducted to assess the efficacy of corneal collagen cross-linking (CXL) in the last decade. The results were promising as collagen cross-linking showed significant improvement in visual acuity and keratometric values. Moreover, initial results show that it is a safe procedure with few reported complications.
Conclusion: CXL is an emerging treatment method in ophthalmology that offers the possibility to effectively treat progressive KCN.

Introduction

Keratoconus (KCN) is a progressive corneal ectatic disorder characterized by bilateral inferior steepening of the cornea. The alteration in matrix collagen production causes an irregular protrusion of the cornea. The specific cause that initiates this disease in not well understood, but it is long known that collagen fibrils play a major role in determining the shape and biomechanical properties of the cornea. Treatment options for patients with KCN include the use of spectacles and contact lenses. For patients who cannot tolerate contact lens wear or do not achieve good vision with contact lenses, the implantation of an intracorneal ring segment (ICRS) may be considered. This improves visual rehabilitation and facilitates the use of contact lenses.[1] In advanced stages however, with corneal scarring or severe thinning, the above-mentioned methods cannot restore good vision, and corneal transplantation like penetrating keratoplasty (PK) or deep anterior lamellar keratoplasty (DALK) may be the only treatment options.[2] Unfortunately, none of the mentioned traditional treatments can alter the natural history of KCN. It was at the late decades of the twentieth century that collagen cross-linking was reported to be of great benefit for stiffening the cornea.[3,4] This new treatment modality offered new hopes to stop the progression of KCN. In this paper we aim to review the most recent publications regarding the application of corneal collagen cross-linking (CXL) in KCN.

Literature search

The literature review was performed using PubMed and Scopus databases on abstracts of articles from 2003 to 2017. The following keywords were used: cross linking, cross-linking, crosslinking, keratoconus, keratectasia. The electronic references were initially scrutinized based on the titles and abstracts. Full text articles were then screened based on their relevance to the subject. Only English written papers were included in this study.

History

CXL is widely used for several applications such as tissue fixation and prosthetic heart valve stiffening.[5,6] Photo-sensitized oxidation in biologic systems was first introduced in 1968 by Foote et al.[7] Then Fujimori et al. explained the photo oxidation of collagen and its cross-linking by either ozone or ultra violet (UV) light.[8] In cornea, cross-linking was introduced in 1998 in Germany by Sporl et al., in porcine eyes.[9] Their results showed the efficacy of riboflavin and UV (365 nm), Glutaraldehyde (0.1%, 10 min) and Karnovsky's solution (0.1%, 10 min) on increased stiffness of the cornea.[3] Later, these three agents were studied in vivo in rabbit eyes and riboflavin − UVA was suggested for studies in human.[10] Wollensak et al. conducted pilot study on humans using riboflavin UVA in 2003.[11] In the United States, the FDA approved CXL in 2016 according to the results of three 12-month clinical trials for treating progression of KCN and post-LASIK ectasia.[12,13] However, a 2015 Cochrane review by Sykakis et al. reported the insufficiency of collected data by published papers to clarify the beneficial utilization of CXL in KCN.[14]

Basic principles of CXL

Collagen plays a supportive role in various human tissues. Inter molecular cross-links between collagen monomers help to strengthen the structure of collagen. Cross-linking happen as a normal process in human cornea as well as during ageing or as a result of diseases such as diabetes. In human cornea, collagen cross-linking process occurs in 3 ways: enzymatic, glycation, and oxidation. The enzymatic cross-linking is part of normal maturation of collagen fibrils.[15] The end of the collagen fibril is not in helical form and contains lysin or hydroxylysine amino acids. Enzyme lysin oxidase would catalyze these residues into aldehyde groups which then reacts with lysine/hydroxylysine residues inside the triple helix structure.[16] The result is binding head to tail through bivalent bond formation which will be converted into trivalent bonds later.[17]

Glycation is a non-enzymatic mechanism with bonding between sugar (advanced glycation end product such as pen-tosidine) and the amino group of a protein.[15] This was called Maillard or Browning reaction.[18] Pentosidin is an advanced glycation end product which is able to form covalent bonds between arginine and lysine residues.[15] This mechanism occurs significantly in diabetes mellitus and to a lesser extent in aging process. Corneal stiffness in older people and slower progression of KCN in diabetes mellitus type 2 has been attributed to this mechanism.[19−21]

CXL takes advantage of oxidation reactions to form bonds between collagen fibrils in the treatment of KCN.[7] Reactive oxygen species (ROS) is generated by UV light and then mediates the process of converting monomers into cross-linked polymers.[15] Oxygen needs to be present in the tissue to participate in this reaction. Richoz and colleagues[22] found that the biomechanical effect of CXL is oxygen dependent and low oxygen tension might reduce the effect of CXL. Kreuger et al.[23] observed that the oxygen is depleted rapidly during CXL, concluding that oxygen and ROS may play an important role in polymerization process.

The technique of cross-linking for keratoconus treatment

Many protocols have been suggested for collagen cross-linking up to now, but the mainstay of all is the same as demonstrated by Wollensak.[11] In sterile settings, after anes-thetizing the eye, the central 8−9 mm of the corneal epithe-lium will be removed and a 0.1% riboflavin solution, consisting of 10 mg riboflavin-5-phosphate in 10 ml dextran 20%, will be instilled for about 30 min (2 drops every 2 min) to the cornea. Riboflavin acts as a photosensitizer and increases UVA absorption by the cornea. After 30 min of instillation, irradiation with UVA of 370 nm and 5.4 J/cm2 is applied for 30 min. During irradiation, riboflavin instillation (1 drop every 2 min) will be continued in addition to balanced salt solution (every 6 min to moisten the cornea).[11,24] It must be noted that irradiation dose should be individualized for every patient to reassure prevention of any harm. De-epithelialization is performed to provide a facilitated homog-enous diffusion of riboflavin inside the cornea.[24] In human studies, the penetrance of riboflavin was limited to the anterior 300 μ even with longer application time and more concentrations.[25]

Other protocols include CXL without de-epithelializa-tion,[26] CXL with riboflavin in a femtosecond laser-created pocket,[27] excimer laser epithelial removal and mechanical full thickness epithelial removal.[28]

Indications for CXL

CXL is meant to stop the progression of KCN. Therefore, the best candidates for this treatment are patients who are suffering from progressive corneal ectasia. Several parameters are proposed to define the progression of corneal ectasia, but in most studies, progression was defined as an increase of 1.00 diopter (D) or more in the steepest keratometry mea-surement, an increase of 1.00 or more in manifest cylinder, an increase of 0.50 or more in manifest refraction spherical equivalent (MRSE) in one year, reduction of central corneal thickness $\geq$ 5% in three consecutive tomographies in 6 months.[29,30]

History of corneal surgery, known sensitivity against in-gredients used during the procedure, corneal pachymetry less

than 400 μ, history of corneal pathology such as herpes simplex keratitis, pregnancy and lactation are considered to be contraindications for CXL.[31]

Efficacy and clinical outcomes of CXL

Several clinical studies have been conducted around the world to assess the efficacy of CXL in KCN. The first clinical study in human eyes was published in 2003.[32] In this non-randomized pilot study, Wollensak and colleagues investigated the efficacy of CXL in 23 eyes. The results of their work showed the stabilization of KCN progression in all eyes. Several clinical studies have been performed after the work of Wollensak. In Siena eye cross study-an Italian non-randomized trial-the effectiveness of cross-linking on stopping the progression of KCN was assessed. The preliminary results of this study showed that CXL improved the uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) of treated patients. Moreover, authors indicated that cross-linking is able to improve the keratometric values and reduce higher order aberrations (HOAs) in keratoconic eyes.[33] The long-term results of Siena eye cross study, confirmed that CXL is useful in inducing a durable stability for KCN.[34] In a larger case-series study, the results of CXL on 142 eyes were reported. Patients were followed for 12 months. The results showed the stabilization and improvement of BCVA in 40.0% of patients after 12 months.

CXL has shown to be efficacious in stopping the progression of KCN on a long-term basis.[34−37] Raiskup and colleagues evaluated the long-term efficacy of CXL in a retrospective interventional study. After 10 years, Kmax and Kmin decreased significantly and BCVA showed a significant improvement. They concluded that CXL can achieve a long-term stabilization of KCN.[38] O'Bart came with the same conclusion after a seven year follow-up.[39]

Theoretically, the UVA light may damage the endothelial cell layer and this is why CXL is contraindicated in corneas thinner than 400 μ. Some studies have reported a decrease in endothelial cell count following CXL, however the reduction in endothelial cells was not statistically significant in any of these studies.[40−43] Table 1 summarizes the method and results of studies that evaluated the outcomes of CXL in KCN.

The use of CXL in treatment of pediatric keratoconus

Management of KCN in children poses several challenges compared with adult KCN, including a higher rate of KCN progression and low tolerance of contact lens wear.[57] Several studies have evaluated the usefulness of CXL to treat pediatric KCN.[58−60] The first study in pediatric KCN was performed by Soeters et al. in a case series of five eyes.[60] The result was stabilization of the keratometric parameters and avoiding corneal transplantation in 4 eyes, and also 3 of these showed significant visual and topographic improvement.[60] The largest pediatrics CXL trial is conducted by Caporossi et al. in a prospective study.[58] 152 eyes of 77 patients with age of 10−18 were treated with CXL. They reported improvement in UCVA

of +0.18 and BCVA of +0.16 Snellen lines along with improvement in K-reading and asymmetry index values.[58] One year later, their team published another study on the subject but with transepithelial approach and they observed KCN instability in patients younger than 19 years. Thus they concluded that transepithelial-CXL should be avoided in this age group.[61]

The study of Ocakhan et al. had the largest follow-up time (48 months) on 40 eyes of 40 patients with age of 10−18.[59] Initial deterioration was detected in the first 6 months but promising continuous improvement during the next 42 months occurred. Also they studied Scheimpflug characteristics of pediatric CXL and reported regularization of the anterior corneal shape.[59]

In contrast to prior studies, Chatzi et al. in a retrospective study on 49 eyes of 42 patients with 3 years follow-up of 11 eyes, reported the initial stabilization but late progression with increase in the mean Kmax during month 24−26 emphasizing the transient effect of CXL in pediatrics.[62] Buzzonetti et al. in a case series study on 13 eyes of 13 subjects with transepithelial CXL and 18 months follow-up; reported worsening in keratometry and HOAs in spite of BCVA improvement.[63] However, Salman et al. in a prospective comparative study of 22 eyes through transepithelial approach documented no evidence of progression of KCN over 12 months.[64]

Recently, Padmanabhan et al. reported long-term results of CXL in pediatric KCN with follow-up beyond 2 years and up to 6.7 years. In most patients CXL was effective in stabilization for longer than 2 years, however, after 4 years few eyes showed reversal of the CXL effect.[65] At the last follow-up, they reported improvement in mean BCVA from 0.33 ± 0.22 to 0.27 ± 0.19 logMAR ($P \leq 0.0001$), reduction in mean topographic astigmatism from 7.22 ± 3.55 to 6.13 ± 3.28 D ($P = 0.0001$), mean flattening of 1.20 ± 3.55 D in Kmax ($P = 0.0002$), and mean corneal thinning of 31.1 ± 36.0 μm ($P < 0.0001$).[65]

Timing of CXL was investigated by Chatzis et al. and McAnnena et al., and both strongly recommended to perform CXL following initial diagnosis as soon as possible.[62,66] It is interesting that neither of the studies investigating the use of CXL in pediatric KCN reported any significant complication such as infection or scarring. Hence, safety of the CXL in pediatrics seems to be equal to the CXL in adult patients.

Accelerated versus conventional CXL in treating keratoconus

As mentioned previously, the standard protocol of CXL is introduced by Wollensak in Dresden in 2003.[11] In the standard protocol, central 7 mm corneal epithelium is removed, and riboflavin 0.1% in 20% dextran would be applied to the cornea.[11] In recent decades, this protocol with many variations in riboflavin preparation or irradiation dosage has been tried to achieve better outcomes with shorter treatment duration. These alternatives are called "Accelerated CXL". In

Table 1
Outcomes reported in literature for conventional CXL.

Authors	Number of eyes	Type of study	F/U	BCVA outcome	K value outcomes	CCT outcome	Change in Endothelial cell density	Comments
Arora[44] et al.	30	Prospective intervention	Up to 12 months	Improved by 0.306 ± 0.15 logMAR	Flat K decreased by 0.9 D ($P < 0.05$)			The K values did not show any significant difference in patients with mean K > 53
Caporossi[34] et al.	44	Prospective intervention	Up to 60 months	Improved by 1.9 Snellen lines	Kmean decreased by 2.0 D			
Touboul[40] et al.	142	Case series	Up to 12 months	The mean BCVA changed from 0.34 ± 0.25 logMAR to 0.33 ± 0.25 logMAR after 12 months	Kmax decreased more than 2.0 D in 21.3%	Mean reduction 11 ± 12	Reduced by 110 ± 82	
Bak-Nielsen[45] et al.	60	Prospective randomized case control	Up to 6 months	Increased from 0.19 ± 0.26 to 0.14 ± 0.18 logMAR	Kmax decreased from 53.1 ± 4.9 to 52.6 ± 5.2			Mechanical compression of cornea did not alter the results of CXL
Caporossi[33] et al.	10		Up to 6 months	Improved 1.66 Snellen lines	Kmean decreased 2.1 ± 0.13	Increased from 431.5 to 450.6	No difference in ECD was observed	
Chang[46] et al.	104 eyes (66 KCN)	Prospective intervention	12 months	Improved 1 Snellen line	Kmax decreased by 1.7 D			
Coskunseven[43] et al.	38	Prospective comparative	Up to 12 months	Improved by 0.1 ± 0.14 logMAR	Not provided	Did not change significantly during f/u	Did not change significantly during f/u	
Rosa[47] et al.	57	Prospective intervention	24 months	Improved by 0.25 ± 0.02	Kmax deceased by 2.22 ± 0.45			
Goldich[48] et al.	17	Prospective intervention	36 months	Did not change	Slight increase in Kmax in 36 months compared with 24 months (52.5 vs 51.7 D)		No change during follow-up	
Greenstein[49] et al.	104 (66 KCN)	Cohort	12 months	Improved by 0.1 logMAR	Kmax decreased 1.0 D			Eyes with a Kmax of 55 or more were 5.4 times more likely to have topographic flattening of 2.0 D or more
Hashemi[50] et al.	40	Prospective case series	60 months	Improved by 0.12 ± 0.08 logMAR	Kmax decreased 0.16 ± 2.20 D Kmean decreased 0.10 ± 1.69 D	The CCT increased from 483.87 ± 29.07 to 485.95 ± 28.43 µm		
Ivarsen[51] et al.	28	Retrospective f/u	Mean f/u of 22 months	No change	Kmax decreased 1.7 D			In 14 eyes Kmax improved more than 2.0 D
Kanellopoulos[52] et al.	231	Prospective intervention study	Uo to 36 months	Improved by 0.20 ± 0.21 logMAR	K 2 decreased 4.41	Thinnest corneal thickness decreased 81.39		

Study	N	Design	Follow-up	Visual acuity	Keratometry	CCT	ECD / Contrast
Khan[53] et al.	71	Prospective intervention	Up to 12 months	Improved 2.37 ± 1.10 Snellen lines in 56.3%	Kmax decreased 2.64 ± 1.42 D in 60.6% patients	Decreased by mean 10.32 ± 21.19 μm	
Kymionis[36] et al.	25	Prospective interventional case series	60 months	Improved from 0.29 ± 0.21 to 0.18 ± 0.18 logMAR	Kmax decreased from 52.53 ± 6.95 to 49.10 ± 4.50 D		Mean endothelial density was 2708 ± 302 cells per square millimeter and did not change significantly during f/u
Lamy[54] et al.	68	Prospective intervention	24 months	Improved 0.16 logMAR	Kmax decreased 1.11 D, Keratometry in the steepest meridian decreased 0.61		Treated eyes showed an improvement of 0.16 Log in contrast sensitivity
O'Brart[37] et al.	30	Retrospective f/u	Up to 72 months	Improved from 0.8 ± 0.27 to 0.905 ± 0.24 logMAR	Kmean improved from 46.44 ± 3.4 D to 45.6 ± 3.3 D		
O'Brart[39] et al.	36	Prospective cohort	94 months	Increased from 0.85 ± 0.25 to 0.96 ± 0.17 Snellen decimal equivalent	Kmax reduced 0.74, Simulated topographic keratometry reduced by 0.74		
Raiskup[38] et al.	34	Retrospective interventional case series	120 months	BCVA improved by 0.14 logMAR	Mean apical keratometry decreased from 61.5 D to 55.3 D, Kmax decreased from 53.2 D to 49.56 D, Kmin decreased from 47.5 D to 45.5 D		
Seyedian[55] et al.	26	Randomized controlled clinical trial	12 months	Improved by 0.13 logMAR in the treated group	Kmax decreased by 0.22 D in treated eyes and increased by 0.41 in the control group		
Vinciguerra[42] et al.	28	Prospective non-randomized study	24 months	Improved from 0.28 to 0.13 logMAR	Kmax decreased from 50.37 D to 49.02 D, Kmin decreased from 46.10 D to 45.43 D		Decreased from 2651/mm² to 2520/mm² (which was not statistically significant)
Viswanathan[56] et al.	51	Prospective interventional study	Up to 48 months	Improved by 0.05 ± 0.13 logMAR in the treated group, decreased 0.05 ± 0.14 (P = 0.2) in the control group	Kmax decreased by 0.96 ± 2.33 D, Kmax increased by 0.43 ± 0.85 D in the control group	Decreased from 470.35 ± 39.26 to 467.64 ± 43.54 (P = 0.6)	
Wittig-Silva[41] et al.	46 treated eyes, 48 control group	Prospective randomized control trial	36 months	Improved 0.09 ± 0.03 logMAR in the treated group	Kmax increased by 1.75 ± 0.38 D in control group, Kmax decreased −1.03 ± 0.19 D in treated eyes	Decreased 19.52 ± 5.06 μm (P < 0.001) in the treated group	Decreased 35 ± 50/mm² (P = 0.490) in the treated group
Wollensak[32] et al.	23	Prospective non-randomized clinical pilot study	Up to 48 months	Improved 1.26 ± 1.5 Snellen lines	Kmax decreased 2.01 ± 1.74 D		The endothelial cell density remained unchanged (P = 0.45)

BCVA: Best corrected visual acuity; CCT: Central corneal thickness; logMAR: Logarithm of minimum angle of resolution; D: Diopter; KCN: Keratoconus; F/U: Follow-up; ECD: Endothelial cell density.

accelerated protocols, high energy setting is applied (up to 30 mW/cm^2) compared with 3 mW/cm^2 in the standard protocol.[67] However, the limit of total dosage should be obeyed as risk of intracorneal complications would significantly increase in doses more than 5.4 J/cm^2.[68] In 2015, Konstantopoulos reviewed the published papers in different protocols.[69] Nine studies with conventional protocol were compared with studies with accelerated protocol. The range of follow-up time in accelerated CXL studies was 6−18 months. They reported that efficacy, visual acuity, and safety were not significantly different between two groups and concluded that the efficacy of accelerated CXL was equal with conventional CXL.[69] No serious complications were reported in three accelerated CXL studies.[70−72] The limitations of these three studies include a short follow-up time and limited number of cases. Waszczykowska et al. followed 16 patients with accelerated CXL (6 mW/cm^2 for 15 min) for 24 months.[73] They reported significant corneal flattening in 18.7% of patients also increased formation of subepithelial corneal haze compared to standard procedure.[73] Shetty et al. published a comprehensive study between four irradiation protocols (3 mW/cm^2, 9 mW/cm^2, 18 mW/cm^2, 30 mW/cm^2) at one year follow-up and reported that the greatest flattening effect was for conventional method compared to the other three accelerated protocols.[74] Wernli et al., tried irradiation intensity of 40−45 mW/cm^2 for 2 min and reported that the corneal stiffness increased compared to the standard protocol, but higher intensities from 50 to 90 mW/cm^2 did not yield in more stiffness.[75] Tomita et al. and Kymionis et al. reported shallower demarcation line in accelerated CXL concluding that accelerated protocol may have less effect compared to the conventional method.[76,77] Besides that, Hammer et al. stated that accelerated CXL may be associated with reduced biomechanical stiffness partly due to insufficient oxygen diffusion into the cornea.[78] However, Tomita et al. and Hashemi et al. reported no difference in keratometry comparing these two groups at one year.[77,79,80] Chow et al. also, reported no inter-group differences in the improvement of UCVA, BCVA, and spherical equivalent between conventional and accelerated (18 mW/cm^2 for 5 min) groups.[81] Aldahlawi et al., measured corneal resistance against enzyme digestion as a kind of corneal stability index after CXL in conventional and accelerated groups and found only minor differences in enzymatic resistance in irradiation range from 3 mW/cm^2 to 18 mW/cm^2.[82] Elbaz et al. and Kymionis et al. compared 9 mW/cm^2 for 10 min with conventional one in a 3 months follow-up.[67,83] Hashemian et al., compared 18 mW/cm^2 for 5 min with conventional CXL in a 6 months follow-up study.[84] Vega-Esterada et al. and Mita et al. compared 30 mW/cm^2 for 3 min with conventional method in a 6 months follow-up study.[85,86] All of these clinical studies confirmed the acceptable efficacy of accelerated protocols. In spite of many studies on accelerated CXL in adults, there were only few in children. Shetty et al., in a prospective study of 30 eyes of 18 patients under 14 years of age, investigated the accelerated CXL in pediatrics.[87] All patients underwent accelerated CXL and were followed for 24 months. They reported improvement in UCVA, BCVA, K1, and K2 with no serious complications, concluding that accelerated CXL is an effective and safe procedure in pediatric KCN.[87]

In a two years follow-up Bozkurt et al. reported improvement in UCVA, BCVA, CDVA, corneal topography readings, total HOA, and coma aberrations with accelerated CXL (30 mW/cm2).[88]

Sadoughi et al. compared the outcomes of the conventional and accelerated CXL and reported the similar refractive, visual, keratometric, and aberrometric results in the two methods but with less adverse effects on the corneal thickness and endothelial cells in accelerated CXL after one year follow-up.[89]

Table 2 summarizes the methods and results of studies evaluating the outcome of accelerated CXL.

Transepithelial CXL compared to conventional epithelium-off CXL

The original method that was employed by Wollensak for cross-linking of cornea included the debridement of epithelium.[32] Recently, a new method of cross-linking has been introduced in the literature that involves the administration of riboflavin and dextran solution on intact corneal epithelium. This method is called transepithelial CXL or TE-CXL.

The removal of lipophilic epithelium would facilitate the penetration of hydrophilic riboflavin molecule through the stroma. Thus, one would assume that the cross-linking effect of TE-CXL would be lower compared to the epithelium-off procedure. However, TE-CXL has gained great popularity and much of recent research in the field of KCN treatment has focused on evaluating the efficacy and safety of this method.

To increase the penetration of riboflavin molecule through the stroma in TE-CXL, some enhancers such as benzalkonium chloride, tetracaine,[90] trometamol and ethylenediamine tetraacetic acid have been introduced. These molecules increase the epithelial permeability of macromolecules through an intact epithelium, without any need for epithelial debridement. The lack of an epithelial defect in TE-CXL may offer faster visual recovery. This method does not need the facilities of an operating room, and the procedure can be performed in less time compared with epithelium-off procedure. This method can be employed for corneas with central corneal thickness (CCT) less than 400 μ.[90−92]

In a study, TE-CXL was shown to be safer than epithelium-off CXL. In the group treated with epithelium-off CXL, transient corneal edema and variable degrees of glare was more prevalent compared with TE-CXL group (80% of cases in epithelium-off CXL versus 0% in TE-CXL group).[93]

The corneal epithelium acts as a barrier for penetration of pathogens and the preservation of corneal epithelium in TE-CXL might decrease the rate of infectious keratitis following CXL.

It has been shown that mean postoperative pain, burning and foreign body sensations was significantly higher in the epithelium-off CXL group than in the TE-CXL group.[94] This has been attributed to exposure of corneal nerves after corneal debridement and release of inflammatory mediators, especially prostaglandins and neuropeptides.[95,96]

Table 2
Reported outcomes for Accelerated CXL in the literature (ACXL: Accelerated CXL, UCVA: uncorrected visual acuity, BCVA: Best corrected visual acuity).

Author	Type of Study	Study features	Results
Waszczykowska et al.	Prospective interventional case series	16 eyes with ACXL (6 mW/cm2 for 15 min) followed for 2 years	- Significant flattening of the cornea in 18.7% of patients with a higher preoperative Kmax value (>50 D) and corneal steepening in patients with a lower Kmax value (<50 D) - Persistent corneal haze in 25% of patients
Shetty et al.	Prospective randomized interventional study	138 eyes with four irradiation protocols (3, 9,18, and 30 mW/cm2) at one year follow up	- Better visual, refractive, and tomographic improvements in the conventional and irradiations of 9 mW/cm^2 and 18 mW/cm^2. - Greater flattening effect in the conventional method
Tomita et al.	Prospective comparative interventional case series	30 eyes with ACXL and 18 eyes with conventional CXL	- Shallower demarcation line in ACXL - Both methods appear to be safe and effective.
Kymionis et al.	Prospective comparative interventional case series	12 eyes with ACXL (9 mW/cm2 for 10 min) and 9 eyes with standard protocol	Deeper demarcation line in the conventional group
Hashemi et al.	Prospective randomized clinical trial	31 eyes with ACXL (18 mW/cm2 for 5 min) and 31 contralateral eyes with conventional method	Comparable in outcome, safety and stopping the progression Better corneal flattening in the conventional method
Chow et al.	Prospective comparative interventional case series	19 eyes with ACXL (18 mW/cm2 for 5 min) and 19 eyes with conventional method	No significant difference in the improvement of UCVA, BCVA, and spherical equivalent
Elbaz et al.	Retrospective comparative interventional case series	16 eyes with ACXL (9 mW/cm2 for 10 min) followed for 12 months	- Improvement in the UCVA - Stabilization of all tested corneal parameters
Kymionis et al.	Prospective comparative interventional case series	10 eyes with ACXL (9 mW/cm2 for 10 min) followed for 3 months	- No endothelial cell loss - No intraoperative or early postoperative complication
Hashemian et al.	Prospective comparative interventional case series	77 eyes with ACXL and 76 eyes with conventional method	BCVA, UCVA, refraction, maximum keratometry, endothelial cell density, anterior and posterior stromal keratocyte density, and subbasal nerve density all were comparable and acceptable in the two groups
Shetty et al.	Prospective comparative interventional case series	30 eyes below 14 years of age with ACXL followed for 24 months	Safe and effective procedure in pediatric patients
Bozkurt et al.	Prospective comparative interventional case series	47 eyes with ACXL (30 mW/cm2 for 3 min) followed for 24 months	Improved UCVA, BCVA, corneal topography, total HOA, and coma aberrations
Sadoughi et al.	Prospective randomized interventional study	15 eyes with ACXL (9 mW/cm2 for 10 min) and 15 contralateral eyes with conventional method	- Similar refractive, visual, keratometric, and aberrometric results - Less adverse effects on the corneal thickness and endothelial cells in ACXL

ACXL: Accelerated CXL; UCVA: Uncorrected visual acuity; BCVA: Best corrected visual acuity.

Soeters et al. reported that in 23% of cases who had undergone a TE-CXL procedure indices of KCN continued to progress after one year. They did not recommend replacing epithelium-off CXL by transepithelial CXL for treatment of progressive KCN.[97] Lesniak et al. reported that the effect of transepithelial CXL on the clinical outcomes of cone flattening (maximum K value) seems to be less than standard CXL (0.9 D vs 1.3 D) at 6 months; however, there was little difference in the visual acuity outcomes.[98]

Caporossi and colleagues compared the clinical results of TE-CXL with epithelium-off CXL in patients younger than 26 years with progressive KCN. 50% of patients were retreated with epithelium-off CXL due to significant deterioration of all clinical parameters after 12 months of follow-up. They offered transepithelial CXL for patients with thin corneas (thinnest point less than 400 mm) and in patients older than 26 years with slowly progressive KCN.[61]

Koppen et al.[99] reported that efficacy of transepithelial CXL is approximately 70% less than epithelium-off CXL in terms of mid-term and long-term functional and biomechanical improvement.

Cerman et al. showed that although transepithelial CXL seems to be less effective in improvement of topographic indices (flat K, steep K, and Kmax), its effect on visual acuity is likely to be similar to that of epithelium-off CXL. Stabilization and/or regression was achieved in 97% of patients in the epithelium-off CXL group and 80% of patients in the transepithelial CXL group.[100]

Aixinjueluo et al. evaluated the clinical results of accelerated transepithelial corneal cross-linking in Japanese patients with progressive KCN and reported no intraoperative or postoperative complication with significant decrease in average keratometry, Kmax, and thinnest corneal thickness and a significant improvement in BCVA.[101] Significant

reduction in corneal astigmatism, Kmax and spherical equivalent with reasonable gain in Snellen's visual acuity in TE-CXL were documented by Ameen.[102]

A randomized controlled trial was designed recently by Rush et al. to compare standard epithelium-off technique versus a transepithelial technique with enhanced riboflavin solution. They reported greater improvement in Kmax in epithelium-off group (−1.52 ± 0.66 D) compared with TE (−0.54 ± 0.58 D) with no statistically significant difference in BCVA improvement between two groups (−0.18 ± 0.09 logMAR in the conventional group versus −0.14 ± 0.08 logMAR in TE group).[103]

Complications of CXL

Corneal haziness

Persistent corneal haze is one of the most frequently reported complications of CXL that can affect visual acuity.[104] It is important to remember that corneal haze after CXL differs from the haze of photorefractive keratectomy (PRK). The haze of CXL is located deeper in the stroma and has a dust-like appearance, while the corneal haze after PRK is found in the subepithelial region and is more reticulated. It is postulated that repopulation of activated keratocytes after the immediate loss of keratocytes following CXL is responsible for haze formation.[105]

The demarcation line that is observed after treating the cornea with standard CXL is thought to represent the depth of CXL treatment and thus shows an efficient biomechanical impact. Corneal haze is believed to occur at the demarcation line.[38]

Scheimpflug densitometry and slit-lamp examination show that haze formation peaks one month after CXL. The corneal haze seems to reach a plateau 3 months after the procedure, afterwards the density of corneal haze begins to decrease.[106]

Raiskup and colleagues evaluated the haze formation after CXL in a retrospective study. They reported that 14 eyes out of 163 eyes (8.6%) developed corneal haziness after one year. Patients with advanced KCN were found to be more prone to haze formation because of lower corneal thickness and higher corneal curvature.[107]

Endothelial cell damage and corneal edema

Thin corneas are at increased risk of endothelial cell damage after CXL, thus CXL is not recommended in corneas with a CCT less than 400 μ.[108] Pachmetry is routinely performed before CXL, to avoid the cytotoxic damage to the endothelium following irradiation.[109,110]

Sharma and associates evaluated the risk of corneal edema after CXL in 350 patients with KCN in a retrospective study. They reported that corneal edema occurred in 10 patients (2.9%). While in 5 patients the edema resolved after 3 months, in the remaining 5 patients edema persisted, requiring a penetrating keratoplasty (PK).[111]

Several mechanisms have been proposed that can predispose the CXL treated corneas to develop corneal edema. These include inaccurate measurement of corneal thickness preoperatively and intraoperatively, inadvertent delivery of energy, corneal dehydration, poor focusing of the light source on the cornea and endothelial diseases like Fuchs endothelial dystrophy.[112−114]

Postoperative corneal infection

Infectious keratitis and corneal ulcers are possible complications of CXL.[115−117] Debriding the corneal epithelium during CXL and the resulting epithelial defect, use of soft bandage contact lens, and topical corticosteroids are risk factors for microbial infection.[118]

Severe keratitis with corneal smear positive for pseudomonas aeruginosa has been reported recently.[119]

One study reported four cases of severe keratitis in a group of 117 keratoconic eyes treated with standard CXL.[120]

Another retrospective study of 2350 patients (1715 conventional CXL, 310 transepithelial CXL, and 325 accelerated CXL) over 7 years (from January 2007 to January 2014) showed four eyes with corneal infiltrates that developed moxifloxacin resistant *Staphylococcus aureus* (MXRSA) infectious keratitis.[121] Kymionis et al. reported a case report of diffuse lamellar keratitis after CXL.[122] Acanthamoeba keratitis and polymicrobial keratitis have also been reported after CXL. Poor contact lens hygiene has been considered a potential risk factor in these patients.[116,117]

Few small patient series described the formation of sterile stromal infiltrates after CXL. There are also reports of peripheral sterile ring infiltrates after CXL which resolved completely after instillation of topical steroids.[123] Immune reaction to staphylococcal antigens has been postulated to be responsible for this complication.[124]

Herpetic keratitis after CXL

Several papers have been published that report development of herpes simplex viral (HSV) keratitis following CXL.[122,125] It has been shown that exposure to UV light can reactivate latent HSV infection.[126,127] Moreover, the epithelial debridement, damage to the corneal nerves and use of topical steroid drops have been proposed as risk factors.[126,127] It has been suggested that in patients with a history of herpetic disease, prophylactic antiviral therapy may decrease the possibility of herpetic keratitis after CXL.[126]

Corneal melting

Corneal melting following treatment of KCN with CXL is a rather rare complication. In 2011, Labiris and colleagues reported a case of corneal melting after CXL for KCN. A young man who underwent an uneventful CXL developed corneal melting and descemetocele, which led to corneal perforation.[128]

Excessive corneal flattening

Another rare complication of CXL for treatment of progressive KCN is excessive corneal flattening. As mentioned previously, CXL normally results in reduction of K-readings, but excessive flattening may lead to corneal thinning. Kymionis reported a case of excessive corneal flattening following treatment of progressive KCN with conventional CXL.[129] During the 5 year follow-up, the patient demonstrated significant corneal flattening (11.1 D change in spherical equivalent) and thinning (from a preoperative value of 464 μm to 243 μm).

CXL combined with other treatments

CXL combination with other techniques was suggested first by Kymionis et al. in 2011, known as 'CXL Plus'.[130] As mentioned before, CXL is commonly used to halt the progression of KCN, but it is not very effective on improving the visual acuity. Hence, alongside maintaining the biomechanics of cornea through CXL, we can proposed alternative treatments to improve visual acuity. The first adjunctive treatment used was PRK.[131,132] PRK cannot prevent the progression but it can effectively correct the irregular astigmatism.[133] PRK and CXL can be performed in a simultaneous or sequential manner; both of them seem to be beneficial,[134,135] but some studies support that simultaneous treatments is superior to the sequential one in terms of visual acuity, change in keratometry, and corneal haze.[136] The other adjunctive is phototherapeutic keratectomy (PTK) which can be utilized in epithelial debridement process of CXL. Kanellopoulos et al. removed epithelium by PTK and then applied topography-guided PRK with mitomycin C (MMC) and finally performed CXL (Athens protocol).[134]

ICRS have proven to be useful in KCN or post-LASIK ectasia patients.[137−139] ICRS pieces flatten the cornea without any tissue removal, but they cannot stop the progression of disease.[140] Studies conducted on combination of Intacs and CXL, have different outcomes; some studies support the subject,[141] while others not.[142,143] On the other side, Keraring and Ferrara Ring were used successfully with promising results.[144] The timing can be sequential or simultaneous as for PRK, both of which seem to have favorable results.[145,146] Phakic intraocular lenses (PIOLs) are another option which is not generally recommended in KCN and post-LASIK ectasia alone, because of progressive astigmatism.[133] CXL plus toric posterior chamber PIOL was successfully tried in high myopic eyes with progressive KCN.[147] Other studies confirmed its outstanding outcomes at 6 months.[148,149] Iris-claw PIOL was tried with similar promising results.[150,151] Combinations of three treatments were also studied with acceptable visual improvement. CXL plus ICRS plus PRK with various protocols were tried,[152−154] and in case of high refractive error (making PRK impossible), ICRS implantation was followed by CXL and then toric PIOL,[152] and all reported positive results.

CXL is a non-invasive procedure that offers new hopes to treat corneal ectatic disorders. It can postpone if not to avoid the need for corneal transplantation in patients with KCN. Moreover, it can improve the functional vision in these patients. Since its introduction in the early 21st century, several modifications including transepithelial CXL and accelerated CXL have been carried out to improve the outcomes of CXL and minimize its adverse effects. However, more studies are needed to evaluate the long-term results of this new modality and elucidate the place of CXL in surgical ophthalmology.

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