Clinical Ophthalmology

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ORIGINAL RESEARCH

Outcomes of Collagen Crosslinking in Patients with Keratoconus and Co-Existent Limbal Stem Cell Deficiency

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Purpose: To analyse the outcomes of collagen crosslinking (CXL) in eyes with progressive keratoconus (KC) and secondary limbal stem cell deficiency (LSCD) caused by vernal keratoconjunctivitis (VKC).

Methods: Patients with progressive KC and VKC having co-existent LSCD who underwent CXL from May 2016 to October 2023 were included. Demographic details, clinical and corneal topography data were collected preoperatively, at 1-months, 3-months, 6-months and 1-year. Details of the CXL procedure and early postoperative complications were noted. Uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA) and topographic indices including flattest keratometry (K1), steepest keratometry (K2), mean keratometry (Km), maximum keratometry (Kmax) and thinnest corneal thickness (TCT) were noted at 1-year and compared with the preoperative data.

Results: Fifteen eyes of 11 patients were included in the study. Partial LSCD was present in 14 eyes and underwent epithelium-off CXL and 1 eye with total LSCD underwent transepithelial CXL. The mean logarithm of the minimum angle of resolution (logMAR) UCVA pre-CXL was 0.67±0.38 which improved to 0.54±0.32 at 6-months (p=0.05) and 0.65±0.35 logMAR at 1-year post-CXL (p = 0.005). Pre-CXL Km was 53.5±4.77 (median-51.95D) which decreased to post-cxl value of 51.72±3.65 at 6-months (p=0.014) and 52.34±3.83 (median-51.3D) at 1-year (p=0.09). Kmax reduced from 63.41±7.11 (median-63.7D) pre-CXL to 60.65±5.31 (median-59.6) (p=0.05) at 6-months and 61.4 ±6.01 (median-61.9D) at 1-year post-CXL (p=0.115). Of the 14 eyes that underwent epi-off CXL, 2 eyes (14.28%) had persistent epithelial defects (PEDs), one of which needed amniotic membrane graft (AMG).

Conclusion: In our series, most eyes with keratoconus having co-existent LSCD had uncomplicated ocular surface healing post-CXL and remained stable at 1-year with significant improvement in visual acuity. Our study shows that CXL can be successfully performed in cases of Keratoconus with co-existing LSCD without any serious complications.

Keywords: keratoconus, limbal stem cell deficiency, collagen crosslinking, vernal keratoconjunctivitis

Introduction

Keratoconus (KC) is a bilateral, progressive corneal ectasia resulting in myopia and irregular astigmatism in the affected individuals.[1] While many risk factors have been described for the occurrence of KC, a recent study showed that vernal keratoconjunctivitis (VKC) increased the risk of developing KC by more than 7 times.[2] Apart from redness, tearing and photophobia, severe itching and consequent eye rubbing is one of the characteristic symptoms of VKC.[3] Eye rubbing is known to be one of the most important causative factors of KC.[4] As such, both KC and VKC are often coexistent and affect similar age groups, particularly children and young adults.[3] Chronic ocular inflammation seen in VKC can adversely affect limbal stem cells resulting in limbal stem cell deficiency (LSCD).

While standard technique of corneal collagen cross linking (CXL), that entails epithelial debridement, has become a mainstay for the treatment of progressive KC, it has not been evaluated in cases of KC that have LSCD secondary to chronic VKC. The closure of epithelial defects is impeded in patients with LSCD[5] and is likely to affect the postoperative course after CXL. In our current study, we have evaluated the outcomes of CXL in patients of KC with coexisting VKC and LSCD.

Received: 12 July 2025
Accepted: 9 September 2025
Published: 12 September 2025

Methods

This was a retrospective observational study conducted at the LV Prasad Eye Institute, Hyderabad. All the included patients had given a written informed consent to use the data for the study. The study was approved by the LV Prasad Eye Institute ethics committee (LEC-BHR-R-004) and complied the Declaration of Helsinki. Patient data was screened from May 2016 to October 2023 to obtain data of 89 eyes of 86 patients that had KC with VKC and LSCD. LSCD was classified as partial, if the palisades of Vogt were unaffected in some areas of the limbus, and total, if the Palisades of Vogt were completely destroyed.[6] Patients who were <16 years of age were subjected to CXL without waiting for progression, while patients >16 years of age were observed and CXL was performed if KC was progressive. Progression in KC was defined as an increase in Kmax by 0.5D in 6 months or 1D in 1 year.[7] Of these, 22 eyes of 16 patients underwent CXL. Patients that completed one year of follow-up were included in the study (15 eyes of 11 patients). Data regarding patient demographics, clinical and topographic data (Pentacam, Oculus Inc), details of CXL procedure and follow-up data were collected through the electronic medical records. All the patients were on treatment for VKC and CXL was done when the disease was well-controlled, such that there were no signs of acute allergy at the time of CXL, regardless of the season in which they were presenting. All the patients had preoperative and post-operative data recorded at 1 week, 1 month, 6 months and 1 year. Complications were noted in the early postoperative period. Uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA) and topographic indices including flattest keratometry (K1), steepest keratometry (K2), mean keratometry (Km), maximum keratometry (Kmax) and thinnest corneal thickness (TCT) were noted at 1 year and compared with the preoperative data. Statistical analysis was done using Graph Pad prism. Student's *t*-test was used to compare the preoperative data with the post-CXL outcomes.

Surgical Method

All the patients underwent CXL by the accelerated protocol (9mW/cm^2 for 10 minutes). Corneal epithelium was debrided over the central 9mm zone in cases of epithelium-off CXL. In cases with extensive conjunctivalisation and vascularisation, epithelium in central clear cornea was debrided. Epithelium was left intact in case transepithelial CXL was done. The cornea was soaked with 0.1% riboflavin every 2 minutes for 20 minutes. Hypotonic riboflavin was used when the corneal stromal thickness was less than 400 microns. Riboflavin 0.25% with benzalkonium chloride (BAK) was used to soak the cornea in transepithelial CXL. The soaked cornea was then exposed to UVA at 9mW/cm^2 for 10 minutes to achieve a surface dose of 5.4 J/cm^2. At the end of the procedure, riboflavin was washed and a bandage contact lens (BCL) (Acuvue, Johnson & Johnson) was placed. This bandage contact lens is made of senofilcon A material and has a diameter of 14 mm. All patients received topical moxifloxacin 0.5% eyedrops 4 times a day for one week, topical carboxymethylcellulose 0.5% 6 times a day and topical loteprednol 0.5% 4 times a day tapering every week for 4 weeks. Patients were reviewed at 1 week after CXL and BCL was removed if the epithelial defect had completely healed. BCL was kept longer in patients that had delayed epithelial healing.

Results

Fifteen eyes of 11 patients were included in this study. There were 8 males and 3 females with a mean age of 18.45 (range: 10–32) years. Partial LSCD was present in 14 eyes while 1 eye had total LSCD. All the patients with partial LSCD underwent epi-off CXL with accelerated 9-minute protocol, while the eye with total LSCD was treated with transepithelial CXL (Figure 1).

The mean logarithm of the minimum angle of resolution (logMAR) UCVA before CXL was 0.67±0.38 which improved to 0.54±0.32 at 6 months (p=0.005) and 0.65 ± 0.35 logMAR at 1 year post-CXL (p = 0.05). The mean pre-CXL BCVA was 0.42±0.33 while the post-CXL BCVA at 6 months was 0.31±0.27 (p=0.05) and at 1 year was 0.23±0.12 (p = 0.005).

Topographic indices were analysed. The mean pre-CXL flattest keratometry (K1) changed from 47.16±14.42D (median=48.9D) to 49.05±3.77D (median=48.05D) at 6 months (p=0.322) and 50.03±3.92D (median=49.25D) at 1 year (p=0.239), while the steepest keratometry (K2) values were significantly decreased from 56.34±4.55D (median=55.65D) pre-CXL to 54.55±3.71D (median=54D) at 6 months (p=0.009) and 54.93±4.06D (median=54.9D) at 1 year post-CXL (p=0.033). Pre-CXL mean keratometry (Km) was 53.5±4.77D (median 51.95D) which decreased to post-CXL value of

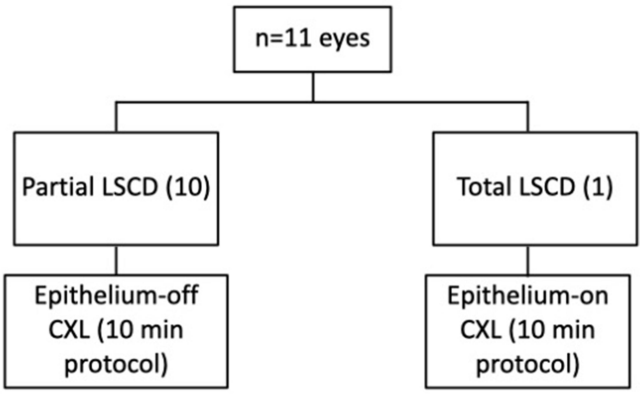

Figure 1 Distribution of cases of limbal stem cell deficiency (LSCD) that underwent collagen crosslinking (CXL).

51.72±3.65D (median=50.87D) at 6 months (p=0.014) and 52.34±3.83D (median 51.3D) (p=0.09). Pre- and post-CXL maximum keratometry (Kmax) at 6 months was 63.41±7.11D (median 63.7D) and 60.65±5.31D (median = 59.6D) which was statistically significant (p=0.05). All eyes were stable at 1 year follow-up after CXL (Table 1).

Two of the 10 eyes with partial LSCD had persistent epithelial defects (PED). One of those eyes healed with extended placement of BCL up to 3 weeks. One eye required amniotic membrane graft (AMG) for the PED 5*3mm in size which did not heal till the one month post-operative visit. After AMG, the eye healed after one week and did not have any other complication. None of the 15 eyes needed limbal epithelial transplant procedures and none of the eyes had infective keratitis (Figure 2).

Table 1 Visual and Topographic Outcomes of Collagen Crosslinking (CXL) in Eyes with Keratoconus (KC) and Vernal Keratoconjunctivitis (VKC) with Coexistent Limbal Stem Cell Deficiency (LSCD)

		Preoperative	Postoperative (6 Months)	p-Value*	Postoperative (1 Year)	p-Value*
UCVA (logMAR)	Mean±SD	0.67±0.38	0.54±0.32	**0.05**	0.65±0.35	**0.005**
	Median	0.6	0.45		0.35	
BCVA (logMAR)	Mean±SD	0.42±0.33	0.31±0.27	**0.05**	0.23±0.12	**0.005**
	Median	0.3	0.25		0.15	
K1 (D)	Mean±SD	47.16±14.42	49.05±3.77	0.322	50.03±3.92	0.239
	Median	48.9	48.05		49.25	
K2 (D)	Mean±SD	56.34±4.55	54.55±3.71	**0.009**	54.93±4.06	**0.033**
	Median	55.65	54		54.9	
Km (D)	Mean±SD	53.5±4.77	51.72±3.65	**0.014**	52.34±3.83	0.09
	Median	51.95	50.87		51.3	
Kmax (D)	Mean±SD	63.41±7.11	60.65±5.31	**0.05**	61.4±6.01	0.115
	Median	63.7	59.6		61.9	
TCT	Mean±SD	400.55±109.11	417.28±47.36	0.258	414±48.28	0.305
	Median	417	418		415	

Notes: *P values calculated between preoperative values and current visit. Bold values indicate statistical significance i.e p-value < 0.05.
Abbreviations: UCVA, Uncorrected visual acuity; BCVA, best corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; K1, flattest keratometry; K2, steepest keratometry; Km, mean keratometry; Kmax, Maximum keratometry; TCT, Thinnest corneal thickness; SD, standard deviation.

https://doi.org/10.2147/OPTH.S548579

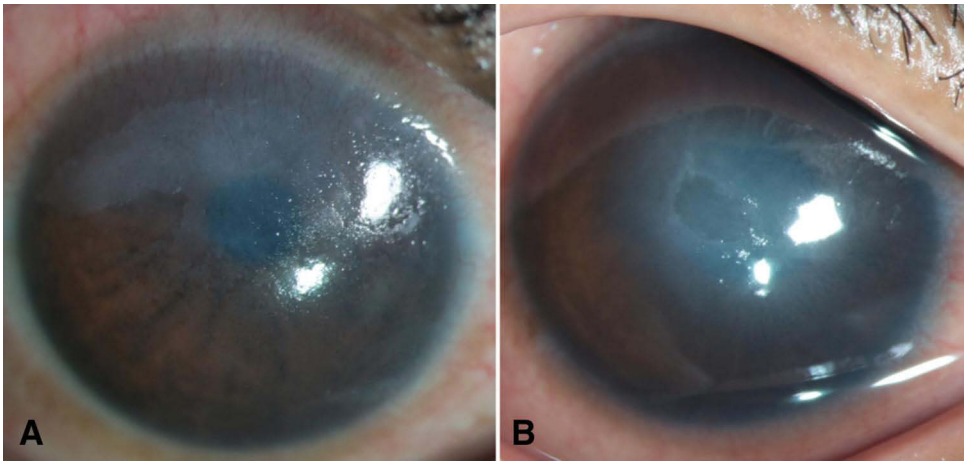

Figure 2 Postoperative ocular surface outcome in eyes with limbal stem cell deficiency (LSCD) that underwent collagen crosslinking (CXL). (**A**) Cornea with completely healed epithelium after CXL (**B**) Persistent epithelial defect (PED) in a case with partial LSCD after CXL.

Discussion

VKC is chronic ocular surface allergic eye disease that, if remains poorly controlled, can cause sterile corneal ulcers, LSCD, keratoconus etc resulting in significant visual loss. Associated with occurrence of keratoconus due to intractable symptoms of itching and eye rubbing, chronic inflammation associated with VKC can also destroy limbal stem cells over a period of time.[8]

VKC is one of the most important risk factors known for KC progression. One of the main symptoms of VKC is itching and eye rubbing which is known to cause KC. An occurrence of Keratoconus that is progressive in such eyes further complicates the matter.[9] A recent meta-analysis reported the risk of developing KC to be 3 times higher among patients with eye rubbing as compared to those without.[4] VKC itself is known to increase the risk of KC by more than 7 times.[2] The prevalence of KC among patients with VKC ranges from 11% to 27%.[9,10] In a study by Cingu and coworkers, more than half the patients of KC with coexisting VKC had grade 4 KC at presentation.[11] This suggests that patients with KC and VKC are more likely to require CXL.

Occurrence of LSCD too is a known complication of chronic VKC. In VKC, chronic Th-2 driven inflammation with eosinophil and mast cell mediators damages the limbal stem cells, while elevated matrix metalloproteinases (MMP)-2 and 9, degrade the basement membrane and extracellular matrix.[10,11] Mechanical trauma from eye rubbing and giant papillae adds to the epithelial injury, and chronic inflammation-driven remodeling of the limbal microvasculature destabilises the limbal stem cell niche.[12] The prevalence of LSCD among patients of VKC has been reported to be 1–2%.[8] Limbal epithelial stem cells play a vital role in maintaining a healthy ocular surface. LSCD causes a breakdown of the barrier function of limbus and consequent conjunctivalisation and vascularisation. Features of LSCD include an unstable tear film, corneal scarring and vascularisation, epithelial defects and irregular epithelium. In partial LSCD, few areas of limbal stem cells are intact resulting sectoral vascularisation of the cornea. While in total LSCD, all the limbal epithelial stem cells are destroyed resulting in complete corneal vascularisation and conjunctivalisation and at times, even corneal melting.[13]

In eyes where there LSCD and Keratoconus that is progressive co-exist, there is always a question of which pathology to treat first, LSCD or progressive Keratoconus? Considering the inherent progressive nature of KC in VKC in our cases, CXL was performed first and patients were followed by for LSCD to treat if needed.

All the patients with partial LSCD underwent an epithelium-off CXL. Transepithelial CXL was done in one eye that had total LSCD. In all eyes a significant improvement in UCVA as well as BCVA was seen at 6 months and 1 year of follow-up. Corneal topography showed a trend towards flattening of the steep keratometry (K2) and the Km and Kmax values over 1 year. Steep keratometry (K2) and mean keratometry (Km) were significantly reduced at 6 months of follow-up. We observed that the Kmean and Kmax were significantly flattened at 6 months, although the decrease did not achieve statistical significance at 1 year. Nevertheless, a trend towards flattening was seen with both the parameters as compared to the preoperative values. We observed an increase in the K1 values over time which could be explained by the effect of corneal remodelling after CXL when a flattening in the cone area is accompanied by a steepening in the

flattest meridian. All the eyes showed topographic stability at 1 year follow-up visit. While the eyes with partial LSCD underwent epithelium-off CXL, the eye with total LSCD underwent transepithelial CXL. Studies have reported transepithelial CXL to be less effective than epithelium-off CXL.[14,15] However, all eyes in our study were stable at one year and showed significant flattening of the steep keratometry values.

Although the outcomes of CXL in paediatric KC with and without VKC are reported to be comparable,[9] a study on more than 2000 eyes reported delayed epithelial healing in patients with KC and VKC undergoing CXL.[16] However, this study did not look at the association of LSCD with delayed epithelial healing in these cases.

PEDs are known to occur in LSCD as epithelial healing is affected in these eyes.[17] Delayed epithelial healing is also known to predispose patients to infective keratitis.[18,19] There are various management strategies described for a PED such as BCL or scleral contact lens, finger-prick blood, autologous serum, amniotic membrane graft (AMG) and limbal epithelial cell transplantation.[20–22] In our series, the incidence of PED was 14.28% (2 eyes of 14). Both the eyes that had PED had partial LSCD since they underwent epithelium-off CXL. The eye with total LSCD had intact epithelium and no postoperative complications were noted. None of the eyes in our series were secondarily infected. One eye with PED was managed conservatively with BCL while the other eye required an amniotic membrane graft (AMG). None of the eyes during the length of follow-up required limbal epithelial cell transplant. Considering that almost 15% of the eyes had ocular surface complications, optimising the ocular surface before proceeding with the CXL and keeping a close watch for epithelial healing issues in such patients is recommended. Another thing that could be considered, would be transepithelial CXL in such eyes. Although we had only one such eye, there were no complications seen in this patient despite having total LSCD. We recommend performing CXL in these eyes without waiting to treat LSCD first, considering the risk of progression of KC in these eyes.

Conclusion

To the best of our knowledge, this is the first study reporting outcomes of CXL in eyes with LSCD secondary to chronic VKC. This being a retrospective study, there were certain limitations. In cases of partial LSCD, we did not have the data on the extent of limbal stem cell loss. It is expected that more the number of clock hours affected, the more the chances of postoperative complications after epithelial debridement. Nevertheless, our study, though having a small sample size, highlights that if closely monitored, majority of the patients with LSCD have uncomplicated ocular surface healing post-CXL. Once the healing is complete, these eyes have a significant improvement in the UCVA and BCVA. Topographic flattening was significant at 6 months in the steepest meridian and all these eyes remained stable at 1 year of follow-up. Since this is a relatively rare condition, our sample size of 15 eyes was considered adequate, though prospective trials on CXL in eyes with LSCD would help shed some more light.

Funding

This study was funded by the Hyderabad Eye Research Foundation (HERF).

Disclosure

None of the authors have any conflicts of interest to declare.

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