

# Collagen cross-linking in keratoconus in Asian eyes: visual, refractive and confocal microscopy outcomes in a prospective randomized controlled trial

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**Abstract** To evaluate the safety and efficacy of collagen cross-linking (CXL) in the treatment of keratoconus. A prospective randomized sham-controlled clinical trial was undertaken and 43 eyes with moderate to severe keratoconus were randomized into two groups that is the treatment ( $n = 23$ ) and the sham ( $n = 20$ ) group. CXL was performed with riboflavin (0.1 in 20 % dextran) followed by UVA radiation (365 nm, 3 mW/cm<sup>2</sup>, 30 min). In the sham group, only riboflavin was administered without UVA radiation. Uncorrected distance visual acuity (UDVA), corrected distance visual acuity, intraocular pressure, corneal thickness, keratometry, endothelial count, confocal microscopy were evaluated at baseline and at 1 week, 1, 3, and 6 months. In cases where CXL was done, UDVA improved by mean  $0.11 \pm 0.06$  logMAR units at 6 months ( $P = 0.01$ ). The refractive cylinder and

spherical equivalent decreased by mean of 0.62 D ( $P = 0.01$ ) and 0.5 D ( $P = 0.19$ ), respectively. Ultrasonic central corneal thickness decreased by mean  $22.7 \pm 10.3$   $\mu\text{m}$  ( $P = 0.01$ ). The maximum and minimum keratometry decreased by mean of  $1.2 \pm 0.8$  D ( $P = 0.01$ ) and  $0.83 \pm 1.2$  D ( $P = 0.39$ ), respectively. The specular count and intraocular pressure did not show any significant change. In the sham group, no significant change was observed in any parameter. Confocal analysis showed that the epithelial healing was complete at 1 week after crosslinking. The sub-epithelial plexus showed loss of nerve plexus at 1 month, regeneration of nerve fibers which started at 3 months and was complete at 6 months. The anterior stroma showed loss of keratocytes with honeycomb oedema and apoptotic bodies till 3 months. The regeneration of keratocytes started at 3 months and was complete at 6 months of follow-up. Collagen cross-linking is an effective procedure to halt progression in keratoconus. The confocal microscopic changes correlate with the outcomes in the treatment and the sham groups.

**Keywords** Keratoconus · Confocal microscopy · Collagen cross linking

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## Introduction

The management options in cases of keratoconus include optical correction with spectacles or contact

lenses, replacement technology with lamellar or full thickness keratoplasty, additive technology with intrastromal corneal ring segments, and strengthening technology with collagen cross-linking (CXL) [1]. CXL of the cornea is a promising new approach to increase the mechanical and biochemical strength of corneal tissue [2]. Cross linking of human collagen is a physiological process and stiffening of the connective tissue is well known in diabetes and aging [3]. The aim of this treatment is to create additional chemical bonds inside corneal stroma by means of highly localized photo polymerization while minimizing exposure to surrounding structures of the eye. Using Ultraviolet-A (UVA) (365 nm), a photosensitizer (riboflavin) is excited into its triplet state to generate reactive oxygen species (ROS). The ROS then react further with various molecules, inducing chemical covalent bonds, bridging amino groups of collagen fibrils (type II photochemical reaction).

Wittig-Silva et al. in a randomized controlled trial showed that in cases where CXL was done, significant reduction in keratometry by 1.45 D occurred in treated cases as compared to controls which showed steepening by 1.28 D after 12-month follow-up [4]. Hersh et al., in a randomized controlled trial, evaluated the safety and efficacy of CXL in keratoconus and corneal ectasia, and found a significant decrease by 1.7 D in the cases at 12 months, and no significant change in the sham and fellow eye controls [5].

We present here the visual, refractive, and confocal microscopy in cases of keratoconus who underwent collagen cross-linking in a prospective randomized controlled trial.

## Methods

We conducted a double-blinded prospective randomized controlled clinical trial at Dr. Rajendra Prasad Centre for Ophthalmic Sciences, a tertiary eye care referral centre at All India Institute of Medical Sciences, New Delhi, India between May 2010 and December 2011.

### Study population

A total of 43 eyes of 42 patients were recruited. All patients more than 14 years of age having keratoconus with Krumeich stage 2 or above grading and

documented progression and decrease in corrected distance visual acuity (CDVA) were recruited. Keratoconus was progressive if there was a subjective deterioration in vision and at least 1 of the following criteria were met over the preceding 12 months: an increase of at least 1 diopter (D) in the steepest simulated keratometry value derived from computerized videokeratography or in the steepest meridian measured by manual keratometry, an increase in astigmatism as determined by manifest subjective refraction of at least 1.0 D or a 0.1 mm or more decrease in the back optic zone radius of the best-fitting contact lens [4].

Written informed consent was taken from all patients. Patients with corneal thickness <400 microns or those with the presence of central corneal scar, history of herpetic eye disease, severe dry eye, corneal infection, history of autoimmune disease, and prior corneal surgery were excluded.

### Randomization

Recruited eyes were labeled from 1 to 43 and randomized into two groups using a table of random numbers. Intervention group underwent CXL and the sham group underwent sham treatment. Randomization of the two eyes was done separately in bilateral cases.

### CXL procedure

The intervention group was treated with CXL within 4 weeks of baseline evaluation. All procedures were performed under topical anesthesia with 0.5 % proparacaine hydrochloride administered twice 15 min before the procedure. A lid speculum was inserted, followed by removal of the central corneal epithelium (7-mm diameter) using a blunt spatula. Commercially available isotonic riboflavin (0.1 % riboflavin with 20 % dextran; Medio-Cross, Italy) eye drops were administered every 5 min for half an hour. The UV device (UV-X, IROC, Zurich, Switzerland) was used to deliver UVA light of 365 nm wavelength and 3 mW/cm<sup>2</sup> irradiance via a 9-mm aperture, at a distance of 5 cm from the apex of the cornea, for 30 min. After irradiation, the eye was rinsed with a sterile saline solution, one drop of moxifloxacin hydrochloride (0.5 %; Vigamox, Alcon Laboratories, Fort Worth, TX) was applied, and a bandage soft

contact lens (PureVision; Bausch & Lomb, Rochester, NY) was inserted and retained for 4 days.

The control group was given sham treatment following epithelial debridement with riboflavin alone, without UVA radiation. The rest of the procedure was similar to that in the treatment group. Post operatively, the patients were prescribed moxifloxacin hydrochloride (0.5 %) three times a day for 1 week, fluorometholone (0.1 %; Flarex, Alcon Laboratories) three times per day for 1 week and preservative free lubricants four times a day for 1 month.

## Evaluation

All evaluation and investigations were done by masked observers. The baseline evaluation parameters included uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), refraction, slit lamp biomicroscopy, fundus examination, Goldman applanation tonometry, Schirmer's test, tear film break-up time, ultrasonic pachymetry (Micropach Model 200P +, Sonomed Inc, Lake Success, NY) for central and 9 point corneal thickness, Orbscan-II (Bausch & Lomb Inc, Salt lake City, UT), non-contact specular microscopy (Topcon SP 3000 P) and confocal microscopy (Confoscan 4, Nidek Inc, Fremont, CA).

The patients were followed at day 1, day 4, first week, first month, third month, and at 6 months. The bandage contact lens was removed on day 4 after complete epithelialization occurred. Investigations were repeated after first week, first month, third month, and at 6 months.

## Statistical analysis

The sample size of the study was calculated as 18 eyes in each group keeping the power as 0.8 and  $\alpha$  as 0.05,

assuming a dropout rate of 10 %, a sample of 20 eyes in each group was chosen. Vision was converted from Snellen fractions to logarithm of minimum angle of resolution (logMAR) units for analysis. Continuous data has been expressed as mean  $\pm$  standard deviation and qualitative data as a percentage. Intergroup analysis was done using the *t* test and Mann–Whitney test for parametric and non-parametric data, respectively. Repeated measure analysis of variance was used to compare the mean values at different time points within the groups. To compare the two groups at different follow-up time points, percentage change from the baseline was computed at each follow-up visit for every patient. Wilcoxon rank-sum test was used to compare the median percentage change between the two groups at each follow-up. The level of statistical significance was kept as  $<0.05$ .

## Results

A total of 43 eyes were enrolled, 23 eyes (22 patients) in the treatment group (cases) and 20 eyes (20 patients) in the control group. The mean age was  $19.7 \pm 5.5$  years in the treatment group and  $21.8 \pm 4.7$  years in the control group ( $P = 0.24$ ). Baseline parameters were comparable in the groups (Table 1). All patients were followed up for a minimum of 6 months and there were no drop-outs.

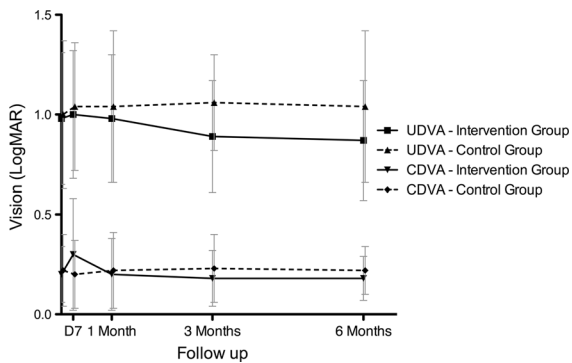
## Visual results

In the intervention group, mean significant improvement of logMAR  $0.11 \pm 0.06$  was seen over 6 months follow-up ( $P = 0.01$ ). At 3 months and 6 months, UDVA was significantly better than controls

**Table 1** Baseline characteristics in the groups

	Treatment group ( $n = 23$ )	Control group ( $n = 20$ )	<i>P</i>
Mean age (years)	$19.7 \pm 5.5$	$21.8 \pm 4.7$	0.24
Gender (M/F)	17/6	11/9	0.68
UDVA uncorrected distance visual acuity, CDVA corrected distance visual acuity, SphEq spherical equivalent, CCT central corneal thickness, $K_{max}$ maximum keratometry, $K_{min}$ minimum keratometry	Mean UDVA (logMAR)	$1.00 \pm 0.37$	0.57
	Mean CDVA (logMAR)	$0.22 \pm 0.18$	0.68
	Mean SphEq (D)	$6.02 \pm 2.53$	0.59
	Mean Cyl (D)	$2.89 \pm 2.11$	0.71
	Mean CCT ( $\mu\text{m}$ )	$448.5 \pm 65.6$	0.59
	Mean $K_{max}$ * (D)	$55.4 \pm 3.8$	0.23
	Mean $K_{min}$ * (D)	$49.3 \pm 2.9$	0.35

\* Orbscan



**Fig. 1** Uncorrected and best-corrected vision in the groups. There was significant improvement in uncorrected vision by logMAR 0.11 ( $P = 0.01$ ) in the intervention group. Significant change was not seen in the control group. UDVA uncorrected distance visual acuity

( $P = 0.03$  and  $0.01$ , respectively). The controls did not show change in UDVA. Worsening of CDVA was seen at 1-week follow-up, probably due to corneal edema ( $P = 0.01$ ). However, there was no significant change with respect to the baseline. The control group also did not show significant change on follow-up (Fig. 1).

#### Refractive results

The mean cylinder in the intervention group decreased from  $2.62 \pm 2.60$  D at baseline to  $2.5 \pm 2.24$  D at 1 week,  $2.5 \pm 1.64$  D at 1 month,  $2 \pm 2.03$  D at 3 months and  $2 \pm 1.79$  D at 6 months. There was significant reduction in the refractive cylinder from the preoperative value by  $0.62 \pm 0.34$  D after 6 months ( $P = 0.01$ ). In the controls, the mean cylinder did not show significant change. The difference between the two groups was significant at 6 months ( $P = 0.01$ ). The mean spherical equivalent in the intervention group decreased from  $6.25 \pm 2.28$  D at baseline to  $5.75 \pm 3.04$  D at 6 months. However, this change was not statistically significant ( $P = 0.19$ ).

#### Pachymetry

The mean ultrasonic central corneal thickness (CCT) in the intervention group was  $466.5 \pm 57.1$   $\mu\text{m}$  at baseline. It showed a gradual decline over follow-up to  $463.7 \pm 31.5$   $\mu\text{m}$  at 1 week,  $461.5 \pm 53.6$   $\mu\text{m}$  at 1 month,  $452.5 \pm 56.2$   $\mu\text{m}$  at 3 months and  $443.8 \pm 54.0$   $\mu\text{m}$  at 6 months. There was significant reduction in the CCT by mean  $22.7 \pm 10.3$   $\mu\text{m}$  at 6 months

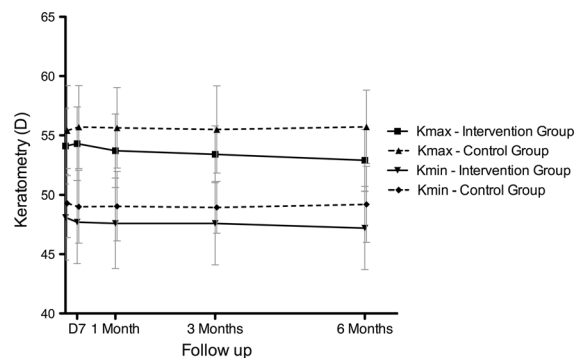
( $P = 0.01$ ). The change in the controls was not significant.

#### Keratometry

The maximum keratometry ( $K_{\text{max}}$ ) in the intervention group, as measured by Orbscan, was  $54.1 \pm 3.2$  D at baseline. It was  $54.3 \pm 3.1$  D at 1 week,  $53.7 \pm 3.1$  D at 1 month,  $53.4 \pm 2.4$  D at 3 months, and  $52.9 \pm 2.6$  D at 6 months. There was a mean regression of  $1.2 \pm 0.8$  D at 6 months that was statistically significant ( $P = 0.01$ ). No significant change was observed in the control group ( $P = 0.34$ ) (Fig. 2). Mean  $K_{\text{max}}$  was significantly less in the intervention group compared to the control group at 3 months ( $2.1 \pm 1.1$  D) and 6 months ( $2.8 \pm 1.3$  D) ( $P = 0.03$  and  $0.01$ , respectively).

The minimum keratometry ( $K_{\text{min}}$ ) in the intervention group as measured by Orbscan was  $48.1 \pm 3.6$  D at baseline,  $47.7 \pm 3.5$  D at 1 week,  $47.6 \pm 3.8$  D at 1 month,  $47.6 \pm 3.5$  D at 3 months and  $47.2 \pm 3.5$  D at 6 months ( $P = 0.39$ ). No significant change was observed in the control group ( $P = 0.87$ ).  $K_{\text{min}}$  was comparable at baseline in the groups ( $P = 0.35$ ) but at 6 months there was significant difference between the intervention group ( $47.2 \pm 3.5$  D) and controls ( $49.2 \pm 3.2$  D) ( $P = 0.03$ ) (Fig. 2).

To determine any complications of the procedure, endothelial cell count, and intraocular pressure were measured at each follow-up visit. These parameters did not show any significant change during the follow-up period ( $P = 0.45$  and  $0.68$ , respectively).



**Fig. 2** Keratometry in the groups on follow-up. Maximum keratometry decreased significantly in the intervention group  $1.2$  D ( $P = 0.01$ ). Significant change was not seen in the control group.  $K_{\text{max}}$  maximum keratometry,  $K_{\text{min}}$  minimum keratometry

## Confocal microscopy

Confocal microscopy examination revealed that the epithelium healing was complete within 1 week after CXL. The epithelium had normal cell mosaic and morphology at 1, 3, and 6 month after the procedure. The sub-epithelial plexus showed loss of the sub-epithelial nerve plexus at 1 month. The regeneration of nerves started at third month with absence of the normal branching pattern and was complete at 6 months with restoration of the branching pattern. Anterior corneal stroma at 1 month showed loss of keratocytes and hypo-reflective honeycomb like lacunar edema and hyper-reflective oval keratocyte nuclei suggestive of apoptotic bodies. At third month there was still some residual edema but keratocyte repopulation had started and activated keratocytes could be seen characterized by enhanced reflectivity. At 6 months, keratocyte repopulation was complete, needle shaped hyper-reflective keratocytes could be seen in anterior stroma and the stromal oedema had completely resolved. The intermediate stroma showed hyper-reflective band shaped striae with hypo-reflective spaces in between suggestive of edema at 1 month. Three-month scan showed repopulation of the stroma by activated hyper-reflective keratocytes and the hyper-reflective striae had disappeared. At 6 months, the repopulation of the keratocytes was complete and the morphology of the keratocytes was restored. No change in the morphology was observed at 1, 3 and 6 month, in the posterior stroma. The morphology of the endothelium was not affected during the follow-up.

In the controls, the epithelial healing was complete at 1-week follow-up with normal cell pattern at subsequent follow-up. The sub-epithelial plexus, stroma and endothelium showed normal morphology at all follow-ups.

## Discussion

We evaluated the visual, refractive, topographic, and confocal results of CXL in adult patients in a randomized sham-controlled clinical trial. Although results of such studies are available for Caucasian eyes, there is no randomized controlled study in Asian eyes. In our patients, uncorrected vision improved by logMAR  $0.11 \pm 0.06$  ( $P = 0.01$ ), mean refractive

cylinder decreased by  $0.62 \pm 0.34$  D ( $P = 0.01$ ), and  $1.2 \pm 0.8$  D regression was seen in  $K_{max}$  over 6 months follow-up ( $P = 0.01$ ). Other randomized controlled trials have published similar findings. In 2008, Wittig-Silva et al. reported preliminary results of the first randomized trial for CXL where they found improvement in vision by logMAR 0.12 and 1.28 D regression in  $K_{max}$  [4]. In another trial conducted by Hersh et al. in 2011, UDVA improved by logMAR 0.12 and there was 2.0 D regression in  $K_{max}$ . In 2012, Lamy et al. demonstrated an improvement in vision by 0.16 logMAR units and 0.99 D regression in  $K_{max}$  [6].

The ultrasonic CCT decreased significantly by  $22.7 \pm 10.3$   $\mu\text{m}$  at 6 months ( $P = 0.01$ ) in our study. We can explain this as the cross-linking process leads to compaction of the corneal stroma. Vinciguerra et al. have also observed that the central corneal thickness decreased significantly at 12 months [7]. In the randomized controlled trial by Greenstein et al., the pachymetric measurements decreased at 1 month (23.8  $\mu\text{m}$ ) and 3 month (7.2  $\mu\text{m}$ ) but recovered by 20.5  $\mu\text{m}$  at 6 months and back to baseline at 1 year [8].

In our study, we found that CXL was a safe procedure with no significant change in endothelial cell count and the intraocular pressure at follow-up visits. There was temporary stromal haze at 1 month in 10 eyes, which disappeared by 3 months, but no permanent vision threatening complications. There are case reports of post CXL infections in the literature including bacterial, herpetic, and acanthamoeba keratitis after crosslinking [9–12]. Notwithstanding these isolated reports, the majority of literature supports our finding that CXL is a safe procedure.

We also found that corneal micro-morphological changes were limited to anterior stroma as seen on confocal analysis. We found that the sub-epithelial plexus regeneration of the sub-epithelial plexus nerve fibres started at 3 months and was completed at 6 months. Other studies by Mazzotta et al., Kymionis et al. and Caporossi et al. showed similar findings [13–15]. The efficacy and benefit of CXL have been demonstrated in various studies over a sustained follow-up period of 6 years [16–18]. Based on the results at the end of our study, CXL was offered to all patients in our control group.

To conclude, we found CXL to be a safe and effective, minimally invasive procedure to halt keratoconus progression.

**Conflict of interest** No conflicting relationship exists for any author.

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