Original Article

Corneal collagen cross-linking with riboflavin and ultraviolet-A light for keratoconus: Results in Indian eyes

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Aim: To assess the results of corneal collagen cross-linking with riboflavin using ultraviolet-A light for keratoconus at one year in Indian eyes.

Materials and Methods: Sixty-eight eyes of 41 patients with progressive keratoconus were included in this retrospective study. All eyes completed was 12 months of follow-up and 37 eyes had a one-year follow-up. The maximum follow-up was 16 months. Ocular examinations including refraction, best corrected visual acuity (BCVA), corneal topography, were recorded at each visit.

Results: The mean age was 16.9 ± 3.5 years (range 12-39 years) and the mean follow-up was 10.05 ± 3.55 months (range six to 16 months). Thirty seven eyes with a follow-up of at least 12 months were analyzed. The preoperative values on the day of treatment were compared with postoperative values of the 12-month examination. This showed that BCVA improved at least one line in 54% (20/37) of eyes and remained stable in 28% (10/37) of eyes (P=0.006). Astigmatism decreased by a mean of 1.20 diopter (D) in 47% (17/37) of eyes (P=0.005) and remained stable (within ± 0.50 D) in 42% (15/37) of eyes. The K value of the apex decreased by a mean of 2.73 D in 66% (24/37) of eyes (P=0.004) and remained stable (within ± 0.50 D) in 22% (8/37) of eyes. The maximum K value decreased by a mean of 2.47 D in 54% (20/37) of eyes (P=0.004) and remained stable (within ± 0.50 D) in 38% (14/37) of eyes. Corneal Wavefront analysis revealed that spherical and higher-order aberrations did not show significant variations in the follow-up period. The coma component showed a very significant reduction at six months after treatment and persisted throughout the follow-up period (P=0.003)

Conclusion: The results show a stabilization and improvement in keratoconus after collagen cross-linking in Indian eyes. This suggests that it is an effective treatment for progressive keratoconus.

Key words: Collagen cross-linking, cornea, progressive keratoconus, riboflavin, ultraviolet-A

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Keratoconus is characterized by the development of a non-inflammatory ectasia of the axial or peri-axial region of the cornea and is usually bilateral. Its incidence in the general population is reported to be about one in 2000.[1] Incidences of one in 600 to one in 420 seem more in keeping with the current diagnostic capacity.[2] Because of the young age of patients, keratoconus often has a significant negative effect on the quality of life.[3]

Two chief mechanisms for the development of keratoconus have been put forward. One proposes that ectasia is closely associated with tissue degradation or reduced maintenance,[4] whereas the other suggests that it is due to slippage between collagen fibrils,[5] with no overall tissue loss. Surgical dissection of the corneal stroma is not resistance-free, even in the posterior region where there is less anterior-posterior interweave, suggesting that there are other elements that bind the collagen lamellae together.[6] Part of this resistance is due to interactions between the collagen fibrils (e.g., Type III and heteromeric Type I and V collagens) and other matrix proteins, such as the proteoglycans,[7,8] and Type VI collagen.[9] In addition, differences in keratocyte surface components, cell morphology and cell-matrix interactions have all been reported in keratoconus.[10,11] If this "interfibrillar glue" were weakened, then lamellae (or collagen bundles) would have the potential to tear apart.[5] The central and inferior regions of the cornea are likely to be affected preferentially (the main region of cone formation), since interlamellar cohesive strength is at a minimum in that area in normal corneas.[12,13]

The technique of corneal collagen cross-linking has been used to at least temporarily block progression of keratoconus in the progressive phase.[14] Cross-linking 'freezes', that is, it arrests the further progression of the corneal collagen thinning/redistribution that is otherwise progressive in keratoconus stromal collagen, increasing the biomechanical stability of the cornea.[15]

The technique of corneal collagen cross-linking consists of photopolymerization of stromal fibers by the combined action of a photosensitizing substance (riboflavin or vitamin B2) and ultraviolet A rays (UVA) from a solid-state UVA source.[14] Photopolymerization increases the rigidity of corneal collagen and its resistance to keratectasia.[16] The cross-linking effect is not distributed homogenously over the corneal depth. The stiffening effect is concentrated in the anterior 200 to 300 microns of the cornea due to the high absorption of UV light in this area.[16]

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The aim of this retrospective nonrandomized open study was to show the results of riboflavin UVA-induced corneal collagen cross-linking in an Indian cohort of patients affected by progressive keratoconus, after one year of follow-up. To our knowledge, this is the first retrospective nonrandomized open label study from the Indian subcontinent.

Materials and Methods

This retrospective nonrandomized open label study with consecutive recruitment comprised patients with the following inclusion criteria:

- Progressive keratoconus
- Corneal thickness of at least 400 μm
- No slit-lamp evidence of corneal scarring

Progression was defined as an increase in maximum keratometry (K) of 1.00 diopter (D) in one year (used from other studies of Caporossi *et al.*, measured in the topography image as the steepest point), patient reports of deteriorating best corrected visual acuity (BCVA)[17,18] (excluding other possible non-cornea-related reasons for deterioration), or the need for new contact lens fitting more than once in two years.[19] Ultrasonic pachymetry was used with 9 points being measured in each eye (PalmScan, Micro Medical Technologies, USA). The apex was measured by collating the cone location magnitude index (CLMI) (Keratron Scout, Optikon, Italy)) from the corneal topography (Keratron Scout, Optikon, Italy). The CLMI is a software built into the Keratron Topographer, and is based on the concept of locating the center of the cone and its magnitude. The algorithm is designed to determine the steepest 2-mm diameter circle within the central 8 mm of the topography. The area-corrected average of all points outside of the circle is subtracted from the corrected average of all the points within the circle. This is repeated for the circle 180° away. The results are compared to decide if the area is a cone.

All patients or the legal guardians (in case of patients less than 18 years of age) provided informed consent after receiving a detailed description of the nature of the treatment. Patients less than 18 years of age were also included in our study as the occurrence of keratoconus is seen at a much earlier age and with rapid progression in the Indian subcontinent.[20] This study was performed in the city of Mumbai in western India.

The cross-linking was performed in the daycare facility of the Clear Vision Eye Center, Mumbai, India. After topical anesthesia of propracaine hydrochloride 0.5% eye drops was administered, the epithelium was removed using a blunt spatula in a 9.0 mm diameter area. This was to ensure that the riboflavin penetrated the stroma and that a high level of UVA absorption was achieved. As a photosensitizer, 0.1% riboflavin solution was applied to the cornea every 5 min for 25 min before the irradiation to allow sufficient saturation of the stroma.[20] Next, an 8.0-mm diameter of central cornea was irradiated with UVA light with a wavelength of 370 nm and an irradiance of 3 mW/cm^2. The CBM (Caporossi, Baiocchi, Mazzotta) X Linker (CSO, Italy), was used as the UVA radiation source. This cross-linker has five pre-focused diodes with a LED divergence of 10° to provide a homogenous UVA radiation. During the 25 min of irradiation,[2] drops of 0.1% riboflavin solution (Ricrolin, Sooft Italia) were applied to the cornea every 5 min to sustain the necessary concentration of the riboflavin.

After the treatment, the eyes were patched for 24 h. On removal of the patches the patients were instructed to use 0.5% moxifloxacin eye drops (Alcon Laboratories, India), 1% prednisolone acetate eye drops (Allergan India) and tears naturale II (Alcon Laboratories, India) for a period of one week. All drops were recommended for use at four times daily.

Follow-up examinations were performed on day 1 and 3 or until complete re-epithelialization. Subsequent examinations were at one, three, six, and 12 months and then advised annually. At each examination, refraction, BCVA (Snellen Vision Charts) with glasses or with contact lenses, corneal topography (Keratron Scout, Optikon, Italy), central corneal thickness (CCT) (PalmScan, Micro Medical Technologies, USA), and intraocular pressure (IOP) (Goldmann applanation tonometer, Haag Streit, Switzerland) were recorded.

To quantify the cross-linking effect, the maximum K value of the apex, maximum and minimum K values in the 3.0 mm zone topography, astigmatism, and BCVA were recorded.

The changes were estimated by subtracting each parameter at the respective follow-up examination from the records prior to the day of cross-linking. Statistical evaluation was performed by analysis of variance (ANOVA) using JMP 4.0 software.

Results

Only patients with a minimum follow-up of 12 months were included in the study. Thirty-seven eyes of 25 patients with a mean age of 16.9± 6.35 years (range 12-39 years) were included. The follow-up ranged from 12 to 16 months. The preoperative apex K value determined with the CLMI was 64.79 ± 7.22 D, the mean maximum keratometry was 53.26 ± 5.93 D (The apex values were determined using the CLMI method used in Keratron Scout, Optikon, Italy and the maximum K was computed using the Simulated K values method. Thus the apex is higher than maximum K), the mean astigmatism was 7.24 ± 4.67 D (this obtained from the topography derived K). BCVA was 0.34 ± 0.30 (vision was recorded by Snellen's and converted to decimal format for statistical calculations). Ultrasonic pachymetery (apex as determined by the topography and approximated to the eye) (Palmscan, Micromed Technologies, USA) was done in all eyes preoperatively to assess suitability and was 478 ± 45 μm.

An evaluation of 37 eyes in which stable parameters were recorded and the preoperative values on the day of treatment were compared with postoperative values of the 12-month examination showed that BCVA improved at least one line in 54% (20/37) of eyes and remained stable in 28% (10/37) of eyes (P=0.006). Astigmatism decreased by a mean of 1.20 D in 47% (17/37) of eyes (P=0.005) and remained stable (within ± 0.50 D) in 42% (15/37) of eyes. The K value of the apex decreased by a mean of 2.73 D in 66% (24/37) of eyes (P=0.004) and remained stable (within ± 0.50 D) in 22% (8/37) of eyes. The maximum K value decreased by a mean of 2.47 D in 54% (20/37) of eyes (P=0.004) and remained stable (within ± 0.50 D) in 38% (14/37) of eyes [Table 1].

Corneal wave front surface aberrometry according to Keratron Scout software (Optikon, Italy) was also used. Spherical and higher-order aberrations did not show significant variations in the follow-up period, whereas the coma component showed a very significant reduction at six months [Fig. 1] with respect to before the operation and persisted throughout the follow-up period (P=0.003).

Table 1: Mean Change in values after cross-linking

Mean ± SD					
Follow-up (months)	**Pachymetry (µm)**	**K max Apex (D)**	**K max (D)**	**Astigmatism (D)**	**Visual acuity**
6	10 ± 7.5	-2.68 ± 8.3	-1.3 ± 4.33	-0.7 ± 3.98	-0.04 ± 0.24
12		- 2.73 ± 7.95	-2.47 ± 3.89	-1.2 ± 4.02	-0.09 ± 0.24

Discussion

Our study reports on a cohort from the Indian subcontinent. To our knowledge this is the first report of its kind. This study like other studies from Europe shows that corneal collagen cross-linking with riboflavin is effective in stopping the progression of keratoconus by "freezing" the cornea.[2,14,19] A good safety profile has been documented.[21] The postoperative change in the keratometry at the apex of the cone (K apex) showed a mean decrease of 2.68 at six months and continued towards reduction at 12 months.

The success of cross-linking treatment in keratoconus is not surprising, because a significantly reduced tensile strength has been measured biomechanically[22] in keratoconus and a significant increase in corneal rigidity has been measured in porcine and rabbit corneas treated by riboflavin/UVA using quantitative biomechanical stress strain measurements.[16,17] Caporossi *et al*.,[2] showed in human eyes that refractive results showed a reduction of about 2.5 D in the mean spherical equivalent, topographically confirmed by the reduction in mean K. Results of surface aberrometric analysis showed improvement in morphologic symmetry with a significant reduction in coma aberrations.[2] In addition, Raiskup-Wolf *et al*.,[19] who followed up patients up to six years and reported on a larger cohort of patients too concluded that the improvement in vision after cross-linking is caused by a decrease in astigmatism and corneal curvature as well as by topographical homogenization of the cornea as a result of the increased rigidity in the cross-linked cornea. In addition, the fitting of contact lenses is improved.[19]

This leads to an increase in both, the unaided visual acuity and BCVA, not only through astigmatism improvement (K readings reduction) but also in terms of corneal symmetry indices improvement after cross-linking. Another possible explanation of cross-linking success, especially concerning keratoconus stabilization, is the new more compact collagen lamellar structure after corneal cross-linking as demonstrated in recent studies by Wollensak[23] and Mazzotta.[24] It is important to note that in this cohort of patients we did not come across any complications.

The importance of cross-linking lies in the fact that it is a low-invasive, outpatient procedure. It achieves a result so far not offered by any other modality of treatment. This includes conservative approaches like contact lenses and surgical options like intracorneal rings, and keratoplasty. Keratoplasty is often the only choice in many patients. In epidemiological studies up to 21% of patients have ended up needing keratoplasty for visual rehabilitation.[25,26] The problems for a treatment like keratoplasty for keratoconus in a country like India are compounded by lack of adequate tissue availability. Also, it is the first treatment option for patients with keratoconus that offers a possibility of mild regression in the condition. Thus cross linking helps in various ways: improves vision, helps regression of disease, stabilizes future progression, and thus probably delays or avoids keratoplasty in a given patient. It would require a longer study to adequately validate these comments.

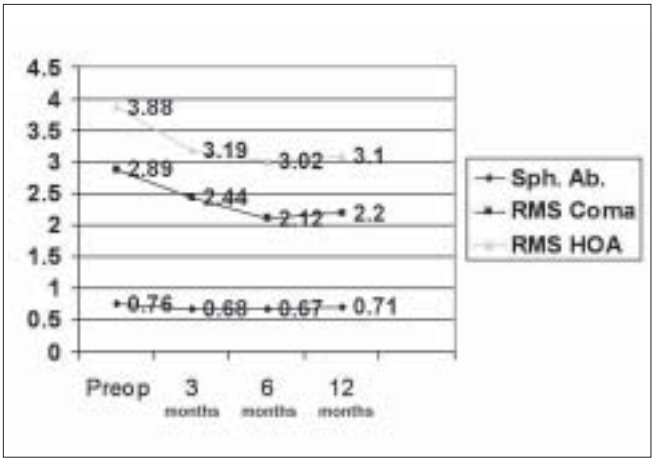

Figure 1: Corneal Wavefront parameters for keratoconus eyes undergoing cross-linking. Spherical and higher-order aberrations did not show significant variations in the follow-up period, whereas the coma component showed a very significant reduction at six months

It thus also has a significant psychosocial value. Keratoconus being a disease of the young causes significant loss of productivity and has a disproportionate impact on the quality of life. Any procedure that can improve the quality of life in a given disease deserves a close look. This study though the first from the Indian subcontinent has several limitations. Being retrospective in nature the data bias is a possibility. However, looking at various international studies published earlier this may not be a major deficit. The numbers in our analysis are limited. A larger multicentric study for collagen cross-linking in keratoconus would be needed to derive stronger conclusions.

At present, keratoconus is not curable. However, cross-linking was able to stop its progression in our series of cases. We have shown improvement in the visual acuity in some cases due to reduction in the irregular astigmatism.

Raiskup-Wolf *et al*.,[19] had two patients in their series who needed repeat treatment with cross-linking. However, these patients had acute exacerbation of neurodermatitis. This study also reported the longest series of patients followed from three to six years after treatment. Thus, it is important to cross-link corneas with progressive keratoconus as early as possible. In the future, we may be able to further improve vision by combining the cross-linking procedure with procedures such as intracorneal ring implantation.[27] and topography-guided photorefractive keratectomy.

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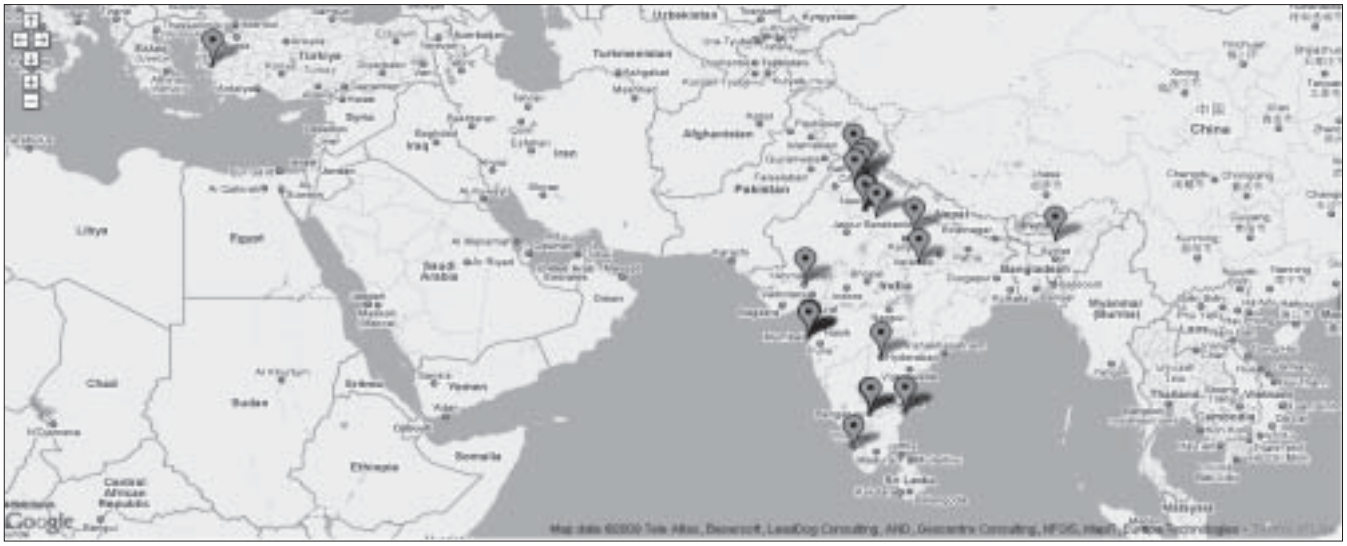

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