Analysis of Two-year corneal cross-linking results in keratoconus patients

Mohamed Iqbal H.

Department Of Ophthalmology, Sohag University Hospital, Faculty of Medicine, Sohag University, Egypt.
Email: dr_m_iqbal@yahoo.com

Abstract: Purpose: To assess and analyze the two-year results of corneal collagen cross-linking with riboflavin using ultraviolet-A light for keratoconus in the treatment of keratoconus and to evaluate the efficacy of this procedure. **Design:** Retrospective study. **Methods:** The preoperative and post preoperative data of 58 eyes of 40 keratoconus patients were revised. The intervention was only conventional corneal collagen cross linking in an indicated keratoconus patient. The data included UCVA, BCVA, slit lamp examination, keratometry, refractometry, pachymetry and corneal topography. Postoperative follow up program was 1,3,6,12,24 months. **Results:** The mean age was 16.9 ± 6.35 years (range 12-39 years) and the mean follow-up was 23.05 ± 1.55 months (range 12 to 30 months). Fifty-eight eyes of 40 patients with a follow-up of at least 24 months were analyzed. The preoperative values on the day of treatment were compared with postoperative values of the 24-month examination. This showed that BCVA improved at least one line in 53.4% (31/58) of eyes, remained stable in 36.2 % (21/58) of eyes (*P*=0.006) and decreased by only one line in 10.3 % (6\58). Astigmatism remained stable (within ± 0.50 D) in 86.2% (50/58) of eyes while decrease by a mean of 1.20 D 13.8 % in (8/58) of eyes. The K value of the apex decreased by a mean of 2.73 D in 65.5% (38/58) of eyes (*P*=0.004), remained stable (within ± 0.50 D) in 25.9% (15/58) of eyes and increase by 1.00 D in 8.6 % (5/58) of eyes. The maximum K value decreased by a mean of 2.47 D in 55.1% (32/58) of eyes (*P*=0.004), remained stable (within ± 0.50 D) in 38% (22/58) of eyes and increase by 1.00 D in 6.9 % (4/58) 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:** This study proved that corneal cross linking is beneficial as both visual preserving and visual improving procedures. K readings are the main indicator of success or failure of the procedure. Central corneal thickness can be an indicator of improvement, there is a reciprocal relationship between them. Best chance is for patients with corneal thickness more than 400 um. It is advised that the crosslinkologist should store the riboflavin in the refrigerator from $+4^0$c to $+8^0$c and discard it immediately after surgery. Use of steroid from the first postoperative day is helpful. Mainly, of visual improvement results from decrease of myopia, yet there was no remarkable improvement of astigmatism.
[Mohamed Iqbal H. **Analysis of Two-year corneal cross-linking results in keratoconus patients.** *Life Sci J* 2013;10(2):2173-2180] (ISSN:1097-8135). http://www.lifesciencesite.com. 304

Keywords: UCVA, BCVA, slit lamp examination, keratometry, refractometry, pachymetry, corneal topography, keratoconus

1. Introduction:

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]

2. Patients and Methods

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

1. Keratoconus grades I or II.
2. Corneal thickness of ≥ 400 µm
3. No previous refractive surgery
4. No corneal scarring.
5. Not pregnant or nursing women.

The surgical procedure

Every patient was subjected to epithelium-off corneal collagen cross linking with the followings:

(I)Preoperative preparation:-

1. Pilocarpine 2 % : 1 drop every 10 minutes ½ hour before surgery to minimize the lens and retina exposure to UV rays.
2. Topical anesthesia : Benoxinate hydrochloride : 1 drop every 5 minutes half an hour before surgery
3. Marking the eye with double check.
4. Skin disinfection: povodine iodine 10% was used to soak the skin.

(II)Device:

- Type: Xlink Opto, Austrilia (Figure 1).
- Parameters:
  - T (Time): 30 minutes.
  - D (Dose): 5.371 J/ccm
  - P (Power): 1.50 mW.
  - I (Intensity): 2.984 mW/ccm

(III)Type of riboflavin:

Riboflavin phosphate 0.127 g (Ricrolin , Sooft) which is equivalent to 0.1% basic riboflavin (Figure 2). Riboflavin was kept in the refrigerator at +4 to + 8C^0 and discarded immediately after surgery.

Figure 1: The device: Xlink, Opto.

Figure 2: Riboflavin phosphate 0.127 g (Ricrolin, Sooft)

Figure 3: 8 mm zone marker used to mark corneal area to be de-epithelized.

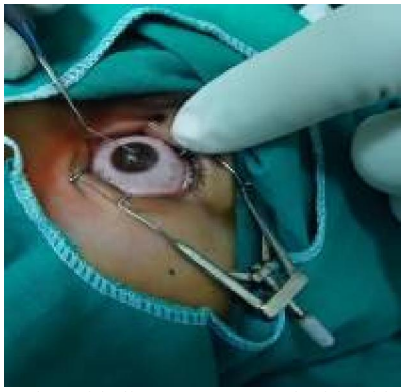


Figure 4: The epithelium was removed with a blunt tipped spatula.

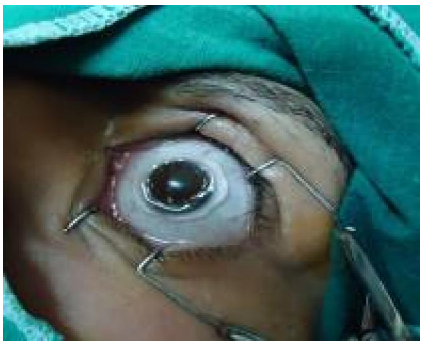


Figure 5: Sodium hyaluronate (Provisc, Alcon) ring to preserve riboflavin on the cornea.

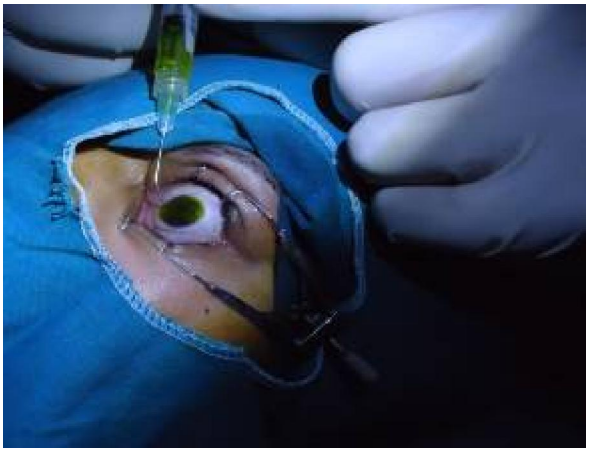


Figure 6: The riboflavin was instilled (while light is turned off).

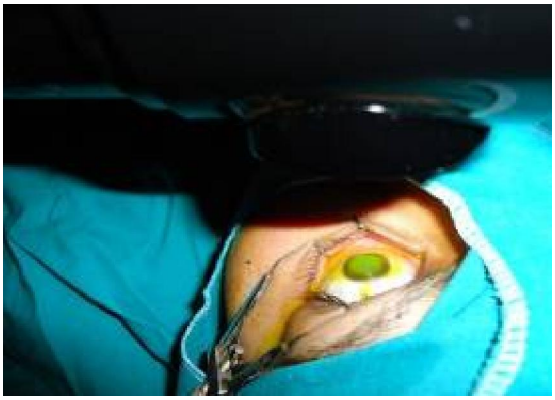


Figure 7: The riboflavin saturated cornea is exposed to UV A rays.

(IV) Operative procedure:

1. 8 mm zone marker was used to mark corneal area to be de-epithelized (Figure 3).
2. The epithelium was removed with a blunt tipped spatula (Figure 4).
3. Sodium hyaluronate (Provisc, Alcon) was applied on the limbus all around to keep riboflavin on the cornea.
4. The lights were turned off in order not to affect the composition and efficacy of riboflavin by the room light (Figure 5).
5. The riboflavin was instilled every 3 minutes for 30 minutes till the corneal stroma saturated with riboflavin.
6. Corneal irradiation with UVA was performed for 30 minutes with dropping of the riboflavin every 3 minutes.
7. Irrigation of the eye was performed.
8. Soft Contact lens was applied onto the cornea.
9. Eye drops were instilled at the end of surgery in the form of :-
    1- Antibiotic: Gatifloxacin 0.3%.
    2- Steroid: Prednisolone acetate 1 %.
    3- Cyclopentolate.
10. Eye patching.

(I) Postoperative treatment: (usually lasts for one week)

1- Antibiotic eye drops :Gatifloxacin 0.3%: hourly during first 24 hours then 4 times daily.
2- Steroid eye drops : Prednisolone acetate 1 %: T.d.s from the 1st postoperative day.
3- Topical gel : twice daily.
4- Systemic Vit. A and Vit. C : twice daily.
5- Systemic analgesic and anti inflammatory.

(II) Postoperative follow up :-

The patient was followed up daily in the 1st week till re-epithelization of the cornea took place. During this follow up, the patient was examined by the slit lamp to detect corneal re-epithelization and haziness. Then the patient was followed up at the 1st, 3rd,6th , 12nd and 24th months postoperatively.

In most cases, re-epithelization took place in 1st 48 hours, then the contact lens was removed and eye patching was stopped. The patient was instructed to wear sun glasses for 2 weeks.

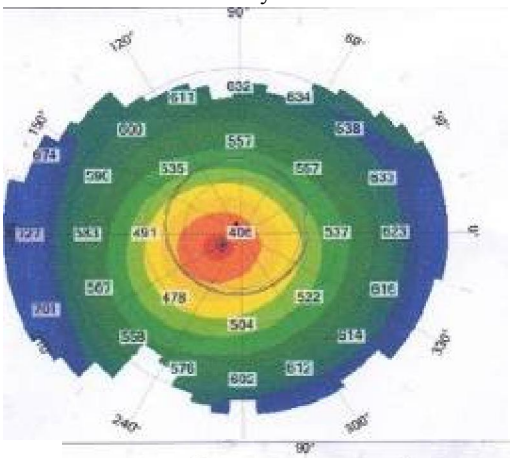
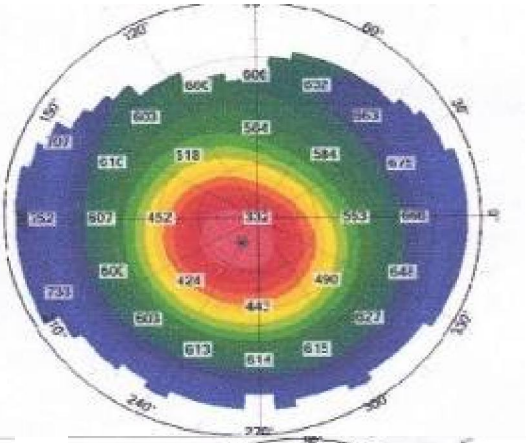
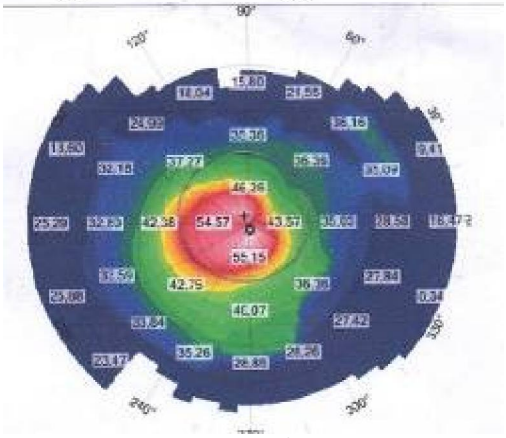
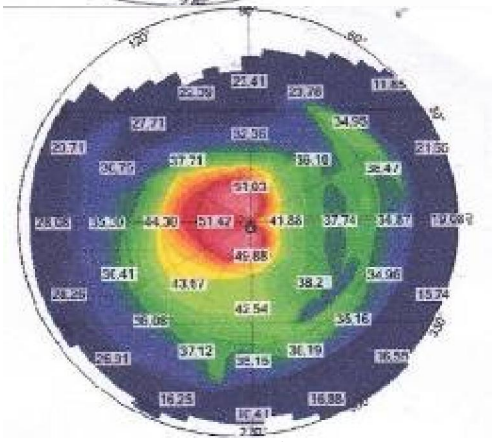
3. Results

The mean age was 16.9 ± 6.35 years (range 12-39 years) and the mean follow-up was 23.05 ± 1.55 months (range 12 to 30 months). Fifty-eight eyes of 40 patients with a follow-up of at least 24 months were analyzed. The preoperative values on the day of treatment were compared with postoperative values of the 24-month examination. This showed that BCVA improved at least one line in 53.4% (31/58) of eyes, remained stable in 36.2 % (21/58) of eyes (*P*=0.006) and decreased by only one line in 10.3 % (6\58). Astigmatism remained stable (within ± 0.50 D) in 86.2% (50/58) of eyes while decrease by a mean of 1.20 D 13.8 % in (8/58) of eyes. The K value of the apex decreased by a mean of 2.73 D in 65.5%

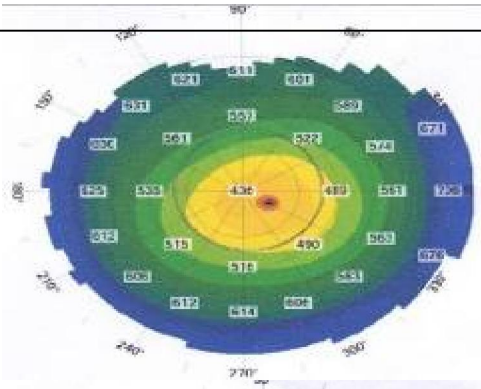
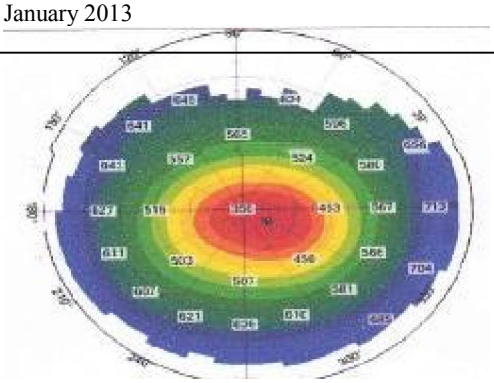
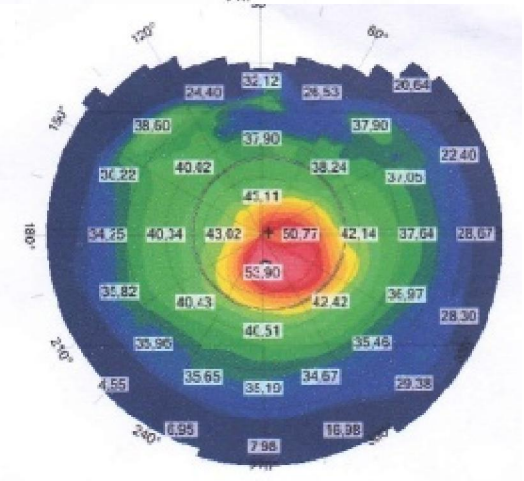
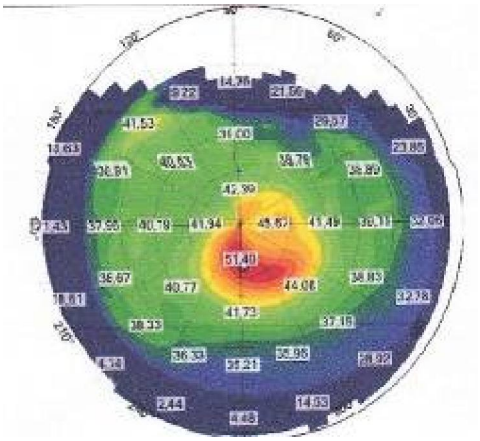
(38/58) of eyes (*P*=0.004), remained stable (within ± 0.50 D) in 25.9% (15/58) of eyes and increase by 1.00 D in 8.6 %(5/58) of eyes. The maximum K value decreased by a mean of 2.47 D in 55.1% (32/58) of eyes (*P*=0.004), remained stable (within ± 0.50 D) in 38% (22/58) of eyes and increase by 1.00 D in 6.9 % (4/58) of eyes. Corneal Wave front 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).

It is important to report examples of the pre- and postoperative patient state:

Example (A) shows the preoperative and postoperative data of one eye

Variant	Preoperative	Postoperative
Date	February 2011	March 2013
Pachymeteric map		
Tangential curvature map		
UCVA	3/60	5/60
BCVA	6/30	6/20
K readings	49.49 @ 18 54.04 @ 108	47.29 @ 8 51.98 @ 98
K Average	51.66 D	49.20 D
Astigmatism	4.55 D	4.69 D
Corneal thickness at the thinnest location	389 Um	308 Um
Corneal thickness at periphery	623 Um	668 Um

Example (B) shows the preoperative and postoperative data of another eye

Variant	**Preoperative**	**Postoperative**
Date	December 2010	January 2013
Pachymetric map		
Tangential curvature map		
UCVA	3/60	4/60
BCVA	6/30	6/25
K readings	46.69 @0 49.02 @ 90	44.13 @ 169 46.62 @ 79
K Average	47.83 D	45.34 D
Astigmatism	2.33 D	2.49 D
Corneal thickness at the thinnest location	423 Um	341 Um
Corneal thickness at periphery	561 Um	567 Um

The results in this study guided me to assume the following hypothesis:

The corneal collagen cross linking leads to corneal rigidity which usually affects the anterior 200-300 um[17-19] of the cornea, however only peripheral cornea increase in thickness while central cornea decrease in thickness which arise many question marks.

This hypothesis is to explain that the central cornea is under 2 antagonist forces:-

A) Pull-back force: (posterior force)

This force pulls the central cornea backwards (posteriorly) by the action of increased peripheral corneal rigidity and thickness leading to decrease corneal curvature (k readings) thus improving the visual acuity.

B) Push- forward force: (anterior force)

This force pushes the central cornea forward (anteriorly) by the action of IOP which is most at the central cornea representing mean IOP vector from the anterior capsule of the crystalline lens to the posterior corneal

surface leading to maximal accumulation of aqueous with forward direction of aqueous currents causing continuous pressure of the posterior corneal surface.

The final result of these 2 forces is that the posterior corneal surface is under 2 hammers:-

1- Rigidity of anterior cornea with continuous pulling back force of peripheral thickened cornea.
2- Continuous pushing forward force of IOP so thinning of central cornea mainly takes place in central posterior cornea.

This study showed that in most cases there was no remarkable improvement in the corneal astigmatism while the basic improvement was the decrease of corneal curvature thus decreasing the myopic element of keratoconus. This may be due to the fact that corneal cross linking affects all meridians symmetrically.

Another hypothesis from this study that needs to be proofed or denied by further studies:-

The optical effects after corneal collagen cross linking is decrease the K readings resulting from:-

1- Decrease corneal curvature leads to decrease radius of curvature and decrease K readings.
2- Decrease central corneal thickness resulting in decrease refractive index of the cornea (bulk loss) leads to decrease K readings.

This combined optical effects of the CXL leads to marked decrease of myopia up to 4 diopters in this study.

4. Discussion

A significant increase in corneal rigidity has been measured in porcine and rabbit corneas treated by riboflavin/UVA using quantitative biomechanical stress strain measurements.[16,20,21] This study reports on a cohort from the Egyptian subcontinent. To my knowledge this is the first report of its kind. This study like other studies from Europe, Asia and India shows that corneal collagen cross-linking with riboflavin is effective in stopping the progression of keratoconus by "freezing" the cornea.[2,14,22,23] A good safety profile has been documented.[24] The success of cross-linking treatment in keratoconus is not surprising, because a significantly reduced tensile strength has been measured biomechanically [25] in keratoconus. 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.

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.*,[22] who followed up patients up to six years and reported on a larger cohort of patients to conclude 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.[22] This leads to an increase in both, the unaided visual acuity and BCVA through 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[26] and Mazzotta.[27] 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.[28,29] 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.

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, although the first from the Egyptian 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 the 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 myopic element of keratoconus.

Raiskup-Wolf *et al.*,[22] had two patients in their series who needed repeat treatment with cross-linking. However, these patients had acute exacerbation of neurodermatitis. That 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.[30] and topography-guided photorefractive keratectomy.

This study showed that in most cases there was no remarkable improvement in corneal astigmatism while the basic improvement was in decrease corneal curvature and decrease myopic element of keratoconus. This may be due to the fact that corneal cross linking affects all meridians symmetrically. On the contrary of other studies, [22,31] we noticed no remarkable improvement in corneal astigmatism & also most cases have regular astigmatism and not irregular astigmatism may be due to strict patient selection to keratoconus grade I and II with corneal pachymetry ≥ 400 um.

5. Conclusion

This study proved that corneal cross linking is beneficial as both visual preserving and visual improving procedures. K readings are the main indicator of success or failure of the procedure. Central corneal thickness can be an indicator of improvement, there is a reciprocal relationship between them. Best chance is for patients with corneal thickness more than 400 um. It is advised that the crosslinkologist should store the riboflavin in the refrigerator at +8 ^{0}c and discard it immediately after surgery. Use of steroid from the first postoperative day is helpful. Most of visual improvement results from decrease of myopia yet no remarkable improvement of astigmatism.

References

1. Rabinowitz YS. Keratoconus. Surv Ophthalmol. 1998; 42:297–319.
2. Caporossi A, Biaocchi S, Mazzota C, Traversi C, Caporossi T. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A rays induced cross-linking of corneal collagen: Preliminary refractive results in an Italian Study. J Cataract Refract Surg. 2006;32:837–45.
3. Kymes SM, Walline JJ, Zadnik K, Gordon MO. Quality of life in keratoconus: The Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study Group. Am J Ophthalmol. 2004;138:527–35.
4. Kenney MC, Brown DJ, Rajeev B. Everett Kinsey lecture: The elusive causes of keratoconus: A working hypothesis. CLAO J.2000;26:10–3. [PubMed: 10656302]
5. Polack F. Contribution of electron microscopy to the study of corneal pathology. Surv Ophthalmol. 1976;20:375–414.
6. Maurice DM. Some puzzles in the microscopic structure of the stroma. J Refract Surg. 1996;12:677–83.
7. Meek KM, Tuft SJ, Huang Y, Gill PS, Hayes S, Newton RH, et al. Changes in collagen orientation and distribution in keratoconus corneas. Invest Ophthalmol Vis Sci. 2005; 46:1948.
8. Scott JE, Haigh M. Proteoglycan-type I collagen fibril interactions in bone and non-calcifying tissues. Biosci Rep. 1985;5:71–81.
9. Hirano K, Kobayashi M, Kobayashi K, Hoshino T, Awaya S. Experimental formation of 100 nm periodic fibrils in the mouse corneal stroma and trabecular meshwork. Invest Ophthalmol Vis Sci. 1989; 30:869–74.
10. Rock ME, Moore MN, Anderson JA, Binder PS. 3-D computer models of human keratocytes. CLAO J. 1995;21:57–60.
11. Yue BY, Baum JL, Smith BD. Identification of collagens synthesized by cultures of normal human corneal and keratoconus stromal cells. Biochem Biophys Acta. 1983;755:318–25.
12. Smolek MK. Interlamellar cohesive strength in the vertical meridian of human eye bank corneas. Invest Ophthalmol Sci.1993; 34: 2962 –9.
13. Smolek MK, Beekhuis WH. Collagen fibril orientation in the human corneal stroma and its implications in keratoconus. Invest Ophthalmol Vis Sci. 1997;38:1289–90.
14. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen cross-linking for the treatment of keratoconus. Am J Ophthalmol. 2003; 135: 620–7.
15. Wollensak G, Spoerl E, Wilsch M, Seiler T. Endothelial cell damage after riboflavin-ultraviolet: A treatment in the rabbit. J Cataract Refract Surg. 2003;29:1786–90.
16. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg. 2003; 29: 1780–5.

17. Kasper M *et al.* Erhohung der Festigkeit der Hornhaut durch vernetzung.[Artificial stiffening of the cornea by induction of intrastromal cross-links]. Ophthalmologe Sporl E, Huhle M. 1997;94:902-6.
18. Kohlhaas M, Spoerl E, Schilde T, *et al.* Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg. 2006;32:279-83.
19. Sporl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye Res. 1998;66:97-03.
20. Kohlhaas M, Spoerl E, Schilde T, Unger G, Wittig C, Pillunat LE. Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg. 2006;32:279–83.
21. Suzuki M, Amano S, Honda N, Usui T, Yamagami S, Oshika T. Longitudinal changes in corneal irregular astigmatism and visual acuity in eyes with keratoconus. Jpn J Ophthalmol. 2007;51:265–9.
22. Raiskup-Wolf F, Hoyer A, Spoerl E. Pillunat LE: Collagen cross-linking with riboflavin and ultraviolet-A light in keratoconus: Long-term results. J Cataract Refract Surg. 2008;34:796–801.
23. Saini JS, Saroha V, Singh P, Sukhija J, Jain AK. Keratoconus in Asian eyes at a tertiary eye care facility. Clin Exp Optom. 2004; 87: 97–101.
24. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA-riboflavin cross-linking of the cornea. Cornea. 2007;26:385–9.
25. Andreassen TT, Simonsen AH, Oxlund H. Biomechanical properties of keratoconus and normal corneas. Exp Eye Res. 1980;31:435–41.
26. Wollensak G, Redl B. Gel eletrophoretic analysis of corneal collagen after photodynamic cross-linking treatment. Cornea.2008;27:353–6.
27. Mazzotta C, Traversi C, Baiocchi S, Caporossi O, Bovone C, Sparono MC, et al. Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: Early and late modification. Am J Ophthalmol. 2008; 146: 527–53.
28. Tuft SJ, Moodaley LC, Gregory WM, Davison CR, Buckley RJ. Prognostic factors for the progression of keratoconus. Ophthalmology. 1994;101:439–47.
29. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. Am J Ophthalmol. 1986; 101: 267–73.
30. Chan CC, Sharma M, Boxer Wachler BS. Effect of inferior segment Intacs with and without C3-R on keratoconus. J Cataract Refract Surg. 2007;33:75–80.
31. Vinay B Agrawal. Corneal collagen cross-linking with riboflavin and ultraviolet-A light for keratoconus: Results in Indian eyes. Indian J Ophthalmol. 2009 Mar-Apr; 57(2): 111–114

5/12/2013