Original Article:

Long-Term Safety and Efficacy of Corneal Cross-Linking in thin Corneas with Keratoconus

Abdulrahman M. Alamri

Associate Professor of Ophthalmology, Department of Surgery,
King Khalid University, Saudi Arabia,
Email: amaamri@gmail.com, Tel. 00966553755973, P.O. Box 641, Abha 61421

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Abstract

Objective: To evaluate the long-term safety and efficacy of corneal cross-linking in keratoconus patients with thin corneas. **Methods:** Forty eyes of 25 subjects having progressive keratoconus with thinnest corneal thickness (TCT) less than 400 µm were evaluated. Uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), corneal topography, and endothelial cell loss were assessed at baseline and followed at six-month interval till the end of 1st year of follow up and then yearly till the fifth year, de-epithelization of cornea was performed, followed by ultrasound pachymetry to measure TCT. Hypoosmolar riboflavin (0.1%) solution was applied after every 2 minutes for 25 minutes. Ultraviolet A (UVA) irradiation was performed for 30 minutes along with riboflavin for every 2 minutes. **Results:** Mean TCT was 381.5 ± 13.2 µm (range: 344–396 µm) without corneal epithelium. Following hypotonic riboflavin instillation, mean TCT increased by 97.8 ± 12.1 µm (range: 83–112 µm) for a mean thickness of 478.1 ± 14.9 µm (range: 409–506 µm). The average follow-up period was 61.79 ± 6.19 months (range: 42–68 months). Corrected distance visual acuity, pachymetry values and posterior elevation showed no significant difference before and after CXL at 5 years follow-up. Corneal astigmatism and maximum keratometry reading (Kmax) were significantly reduced. Mean reduction of endothelial cell count was 1.5% at the last follow-up visit. **Conclusions:** CXL with UVA and hypoosmolar riboflavin was effective in stabilizing keratoconus progression in patients with thin corneas however further studies are required to confirm our results.

Keywords: corneal cross-linking, keratoconus, riboflavin, thin cornea

الملخص

الهدف: تقييم سلامة وفعالية عملية تصليب القرنية المخروطية ذات الترقق على المدى الطويل.

منهجية الدراسة: تم تقييم أربعين عينا من ٢٥ شخصا والذين يعانون من القرنية المخروطية التقدمية حيث كانت أنحف ثخن قرنوي أقل من ٤٠٠ ميكرون. تم تقييم حدة البصر البعيد غير المصحح وكذلك حدة البصر البعيد المصحح وتضاريس القرنية وفقدان الخلايا البطانية في الزيارة الأولى وتلا ذلك في فترة ستة أشهر من المتابعة حتى نهاية السنة الأولى ومن ثم سنويا حتى السنة الخامسة، تم إزالة ظهارة القرنية، تلاها قياس الموجات فوق الصوتية لقياس الثخن القرنوي. تم استخدام قطرات ريبوفلافين ناقص التوتر (٠,١٪) بعد كل دقيقتين لمدة ٢٥ دقيقة ثم التشييع بواسطة الأشعة فوق البنفسجية A لمدة ٣٠ دقيقة مع استخدام الريبوفلافين كل دقيقتين.

النتائج: كان متوسط أنحف ثخن قرنوي ٣٨١,٥ ± ١٣,٢ ميكرون (المدى: ٣٤٤-٣٩٦ ميكرون) دون ظهارة القرنية. بعد تقطير ريبوفلافين ناقص التوتر، كان هناك زيادة في الثخن القرنوي بمقدار ٩٧,٨ ± ١٢,١ ميكرون (المدى: ٨٣-١١٢ ميكرون) لمتوسط الثخن القرنوي ٤٧٨,١ ± ١٤,٩ ميكرون (المدى: ٤٠٩-٥٠٦ ميكرون). وكان متوسط فترة المتابعة ٦١,٧٩ ± ٦,١٩ شهرا (المدى: ٤٢-٦٨ شهرا). لم يظهر اي فرق كبير قبل وبعد تصليب القرنية لكل من حدة البصر المصحح، الثخن القرنوي والارتفاع الخلفي بعد ٥ سنوات من المتابعة. تم تقليل اللابؤرية قرنوية المنشأ وكذلك تقوس القرنية بشكل ملحوظ. وكان متوسط انخفاض عدد الخلايا البطانية ١,٥٪ في آخر زيارة متابعة.

الخلاصة: تصليب القرنية بواسطة ريبوفلافين ناقص التوتر كان فعالا في تحقيق استقرار القرنية المخروطية في المرضى الذين يعانون من ترقق قرنوي ولكن هناك حاجة إلى مزيد من الدراسات لتأكيد نتائجنا.

كلمات مفتاحية: تصليب القرنية، القرنية المخروطية، الريبوفلافين، ترقق القرنية

INTRODUCTION

Keratoconus is a common bilateral, non-inflammatory, degenerative disorder of the cornea with an incidence of 1 in 2000 individuals in the general population.[1] The characteristic feature of this disease is progressive thinning and ectasia of the cornea [2] leading to corneal steepening, irregular astigmatism and reduction in visual acuity.[3] Onset of keratoconus occurs typically at puberty, then progresses for next 10 to 20 years and finally tends to stabilize.[3] Disease symptoms are highly variable depending on the severity and have no well-described signs at the early stage, whereas, in advanced stages, the vision undergoes significant distortion along with visual loss, severe pain, and corneal scarring.[1] The molecular mechanisms governing the pathogenesis of the disease is not yet clear. However, it seems that keratoconus is the eventual manifestation for several conditions, such as reduced number of collagen cross-links leading to decreased biomechanical stability, higher pepsin digestion than in normal corneas, slippage of collagen lamellae, and the loss of normal interwoven lamellar structure.[5]

Fortunately, the conventional treatment options for keratoconus which include, use of rigid contact lens, intracorneal ring segment implantation for early to moderate stages and lamellar keratoplasty or corneal transplantation for advanced stages have been encouraging [6] since, they only improve the visual acuity, but cannot arrest disease progression.[5]

Recently, a novel, minimally invasive technique known as Corneal Collagen cross-linking (CXL) with Ultraviolet A (UVA) has been introduced as a treatment option for progressive keratoconus.[7] CXL improves corneal rigidity by increasing the biomechanical stability of the stromal tissues and also increases corneal resistance to enzymatic digestion, hence arresting disease progression.[7, 2] However, one major limitation of CXL is that it is not effective in thin corneas (< 400µm) due to a risk of corneal endothelial cell damage by UV rays. In fact, in many advanced cases, patients are often excluded from CXL as their corneal thickness is less than 400 µm. In order to overcome this problem, Hafezi et al,[8] proposed an alternative protocol using UVA along with hypoosmolar riboflavin solution. In patients with thin cornea, riboflavin actually helps to swell corneal stroma and increases its thickness before CXL.[8] In fact, riboflavin (Vitamin B2) performs two important functions, firstly, it acts as a photosensitizer, leading to the formation of new covalent bonds between collagen molecules, fibers, and microfibrils by photosensitized oxidation in combination with UVA,[9] and secondly, it protects the deeper ocular structures, such as the corneal endothelium, lens, and retina by absorbing the UVA.[5] This approach of CXL using hypotonic riboflavin actually aids in stabilization of keratoconus with no major complications. Nonetheless, little is known about the long-term safety and efficacy of the treatment.

The present study investigated the long-term safety and efficacy of CXL using UVA and hypoosmolar riboflavin solution for the treatment of kera-

toconus in patients with thin cornea.

Subjects and methods

Subjects

A total of 40 eyes of 25 subjects (18 males, 7 females) with an average age and disease duration of 29.21 ± 5.6 years and 42.1 ± 9.7 months respectively were enrolled in this study. Inclusion criteria involved documented progressive keratoconus, as confirmed by the evaluation of the anterior, posterior elevation maps and keratometry (Kmax) maps, as well as the corneal thickness maps at the thinnest point (obtained using the Pentacam tomography). The average follow-up was 61.79 ± 6.19 months (range: 42–68 months). Exclusion criteria were a history of herpes keratitis, corneal scarring, severe dry eyes, and any autoimmune disease. The protocol was reviewed and approved by Magrabi Aseer institutional Review Board. The study conducted adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all study participants, following a conversation about the nature and risks/benefits of participation.

Methods

Topical anesthesia Alcaine® 0.5% (proparacaine hydrochloride ophthalmic soe lution, Alcon, USA) eye drop was administered to control ocular pain and then 9 mm of epithelial tissue was mechanically removed using a Beaver blade. De-epithelization was followed by measuring thinnest corneal thickness (TCT) via ultrasound pachymetry (Corneo-Gage Plus, SonoGage, Inc., Cleveland, OH, USA) and instillation of hypo-osmolar riboflavin (0.1%) solution to the cornea every 2 min for 30 min. The corneal thickness was checked continuously by ultrasound pachymetry and riboflavin was administered until the corneal thickness reached 400 µm. The center of the cornea was then exposed to UVA light of 370 nm wavelength and irradiated with an energy dosage of 3mW/cm^2 for 30 minutes. During UVA irradiation period, hypo-osmolar riboflavin solution was applied every 2 min to maintain the necessary concentration of riboflavin and to protect cornea from drying up.[7] Following the procedure, topical VIGAMOX® (0.5% Moxifloxacin HCl ophthalmic solution, Alcon Lab. Inc., Fort Worth, TX, USA) and Vexol® (1% Rimexolone Eye Drops, Alcon Laboratories, TX, USA) were administered in all patients until re-epithelialization of the cornea was completed. Rimexolone was prescribed for over four week's periods.

Examinations

Patients were followed at 6 six months interval till the end of the first year of follow up and then yearly till the fifth year. At each examination, BCDVA, corneal tomography (OCULUS-Pentacam®, Wetzlar, Germany), and corneal endothelial cell density (ECD; EM-3000 specular microscope, Tomey, Nagoya, Japan) of each subject were measured.

Evaluation

Paired *t*- test with SPSS software version 20 (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation at baseline and at 6 months interval up to 1 year, and then yearly for 5 years after corneal CXL using the procedure. Statisti-

cal significance was defined as $P < 0.05$.

Results

In the present study, 40 eyes of 25 patients with progressive keratoconus were examined via corneal tomography, preoperatively and at 6 months interval up to 1 year, and then at 1-year interval up to 5 years after treatment. 15% of the patients (6 out of 40 eyes) showed mild corneal haze following CXL procedure, but rest of the patients showed clear corneas after CXL. About 12.5% cases (5 out of 40 eyes) showed development of dry eyes after the treatment.

Corneal thickness: The mean TCT of all eyes with epithelium was 432.5 ±15.7 um. Following corneal epithelial removal, all eyes had a TCT <400 µm and the mean value of TCT was 381.5 ± 13.2 µm (range: 344–396 µm). After the application of hypotonic riboflavin solution, the mean TCT increased to 478.1 ± 14.9 µm (range: 409–506 µm). At 6 months follow-up TCT reduced to 371.12 ±14.2 (p value <0.05) however it stabilized to 382.15±12.9 at the last follow-up (p-value >0.05). This finding agreed with the finding by Coskunseven et al. 12 Table 1 summarized all the preoperative and postoperative values.

Visual acuity: Mean CDVA value (decimal scale) at preoperative stage was 0.7 ± 1.92 and at postoperative stage was 0.7 ± 1.88 (all with p> 0.05) (Table 1). Hence, CDVA showed no significant change in values from pre-CXL to the follow-up visits. Moreover, none of the eyes lost any line of CDVA after the treatment. Additionally, UDVA also remained almost same at pre-operative (0.05 ± 3.51), 6 months, 12 months, and at the last post-operative follow-ups (0.05 ± 3.65).

Corneal topography: Analysis of corneal topography data revealed that the mean Kmax value was reduced by 1.4 Dioptors (D) from the pre-operative value. The pre-CXL Kmax was 62.71 ± 4.68D and the post-CXL Kmax was 61.31± 4.62 D, p <0.05. The K average (Kave) was also decreased by a mean of 0.7 D from pre-operative to 5 years follow-up evaluation from 49.15 ± 2.61 to 48.45± 2.69 D, p <0.05.

Mean Anterior elevation at the thinnest location was significantly reduced to a value of 27.12± 4.89 D post-operatively from 38.12 ± 4.65 D pre-operatively. On the other hand, posterior elevation did not change considerably ranging between 73.48 ± 12.51 D pre-operatively to 72.59 ± 13.29 D postoperatively (p > 0.05). The mean value of corneal astigmatism showed a significant decrease from 5.49 ± 3.16 D before treatment to 4.19 ± 3.22 D (p< 0.05) at 5 years after treatment. Mean endothelial cell density (ECD) was not substantially lost before and after CXL treatment as evident from the values such as: 2789 ± 154 cells/mm^2 pre-CXL and 2748 ± 171 cells/mm^2 at 5 years post CXL.

Apart from corneal haze, slight dryness of eyes, and mild reduction of ECD, no other direct or primary complications of the procedure was reported.

Table 1 Mean preoperative and postoperative results

Parameter	Preoperative	6 months	12 months	Last follow-up
UCVA (Decimal scale) Mean±SD P value	0.05 ± 3.51	0.05 ± 3.78 > 0.05	0.05 ± 3.63 > 0.05	0.05 ± 3.65 > 0.05
CDVA (Decimal scale) Mean ± SD P value	0.7 ± 1.92	0.7 ± 1.83 > 0.05	0.7 ± 1.9 > 0.05	0.7 ± 1.88 > 0.05
K max (D) Mean±SD P value	62.71 ± 4.68	63.15 ± 3.19 < 0.05	62.28 ± 4.74 < 0.05	61.31± 4.62 < 0.05
Corneal astigmatism Mean±SD P value	5.49 ± 3.16	4.14 ± 4.81 > 0.05	4.79 ± 3.48 > 0.05	4.19 ± 3.22 > 0.05
Average Sim K (D) Mean±SD P value	49.15 ± 2.61	50.21 ± 3.73 > 0.05	49.87 ± 2.98 < 0.05	48.45± 2.69 < 0.05
Anterior elevation Mean±SD P value	38.12 ± 4.65	31.54 ± 3.22 > 0.05	29.14± 4.11 > 0.05	27.12± 4.89 > 0.05
Posterior elevation Mean±SD P value	73.48 ± 12.51	72.63 ±13.29 > 0.05	73.85 ±14.83 > 0.05	72.59 ± 13.94 > 0.05
Thinnest corneal thickness (µm) Mean±SD P value	381.5 ± 13.2	371.12 ±14.2 < 0.05	383.55± 14.7 > 0.05	382.15± 12.9 > 0.05
ECD (cells/ mm2) Mean±SD P value	± 2789 154	± 2677 162 0.05>	± 2681 167 0.05>	± 2748 171 0.05>

Abbreviations:
UVA, Uncorrected visual acuity;
CDVA, Corrected distance visual acuity;
K max, Simulated maximum keratometry **values;**
Average Sim K, Average of simulated keratometry **values;**
ECD, Corneal endothelial count density

Discussion

Collagen CXL with UVA and riboflavin is a promising therapeutic method widely performed to strengthen the cornea of patients with progressive keratoconous and to stabilize the disease progression. Wollensak *et al.* found the reaction between riboflavin (a photomediator), oxygen and UVA light of wavelength 370 nm (absorption maximum of riboflavin) resulted in increased corneal stiffness of rabbit and porcine eyes..[4] However, corneal epithelium is impermeable to riboflavin, hence epithelial debridement is normally performed to allow sufficient penetration of riboflavin to corneal stroma in standard 'epithelium off' CXL method.[10] Baiocchi *et al* showed that in absence of riboflavin, around 30% of UVA light is absorbed by the lamellae of intact cornea..[11] On the contrary, in presence of riboflavin, approximately 95% of UVA is absorbed in the cornea, resulting in a 20-fold decrease of the original irradiance, which in turn minimizes the possibility of damage to the endothelium, lens and retina.[12, 7]

The present study showed a significant reduction in TCT at 6 months follow-up (371.12 ±14.2). This finding agrees with that by Gu et al.[2] However the TCT values returned to the pre-operative values at the last follow-up. Besides, it was observed that treatment efficacy of CXL with riboflavin performed on eyes with thinner corneas (<400 µm following epithelial removal) was like the traditional CXL performed on eyes with thicker corneas.

Corneal topography (Kmax and Kave) is considered as one of the key outcome mea-

sures for CXL method.[6] Changes in these measurements provide a more comprehensive analysis of the probable improvement in the shape and optical properties of the cornea after crosslinking. Generally, the topography indices are higher than normal in patients with keratectasia. Thus, a substantial reduction of any of the postoperative measurements after CXL may indicate improvement in the contour of the cornea and increased visual acuity.[13] Several previous reports revealed a Kmax reduction of 1-2 D after 1-year post CXL such as, Henriquez et al reported a Kmax reduction of 2.66 D, Hersh et al reported a decrease of 1.70D, Chunyu et al detected a small change in both Kave (0.4 D) and Kmax (0.26 D) values after 18 months post –CXL.[6] Our experimental results also showed a significant Kmax reduction of 1.4 D and Kave reduction of 0.70 D at 18 months post-CXL. This reduction of Kmax and Kave values could be due to the rearrangement of corneal lamellae and surrounding matrix. However, the duration of the turnover rate of stromal collagen fibers is several years,[6] indicating the necessity of long term follow-up to determine whether repeated CXL treatment is required.

The current study examined the anterior and posterior elevation of cornea because they play an important role in determining keratoconus progression. Both front and back elevation at the thinnest point of the cornea was measured via Pentacam tomography. Anterior elevation showed a significant reduction, whereas the posterior elevation displayed a slight reduction after 5 years post-CXL. Based on our observation, it can be suggested that assessment of elevation is a better way to reveal the long-term effects of CXL and improvements in corneal shape.[3]

Regarding the mean UDVA and CDVA values, our study could not detect any significant changes at pre and post-CXL treatment. Irrespective of the finding, the visual acuity was stable during the follow-up examinations. The same issue is seen with the study of Chunuya et al,[6] where no significant change in UCVA was detected 18 months post-CXL. To display a meaningful correlation between topographic changes and postoperative visual acuity, further research is needed to detect baseline characteristics and outcome measures as potential indicators for visual acuity improvement after CXL.[13] Keratoconus may often lead to corneal astigmatism, a common refractive abnormality that arises due to rotational asymmetry of corneal curvature. CXL is considered to be an emerging treatment for corneal ectasia. Our study demonstrated a decrease of mean corneal astigmatism by 1.3 D at 5 years follow-up post-CXL, indicating the importance of CXL-based corneal treatment for astigmatism correction.

Previous studies showed that corneal ECD was not compromised following either traditional CXL[6,14,15,16] or contact-lens assisted CXL. However, we noticed 1.5 % reduction in mean ECD at 5 years follow up, which can be related to aging.

Development of corneal haze is one of the potential complications of CXL, affecting 10-90% of patients. Corneal haze

may affect CDVA, and hence, deteriorates the quality of vision.[17] In the present study, although mild corneal haze was observed in 15% cases (6/40), yet the values of both CDVA and UCVA remain stable after CXL. Furthermore, the patients with corneal haze did not show any other complications and quality of vision was not found to be affected. Future studies should be done to detect the impact of haze development after CXL.

The main limitation of the present study is the relatively small sample size. Further studies with a larger number of patients should be done for better understanding of clinical outcomes of CXL. Additionally, increased sample size will help to detect whether any difference in complication rates exists between isotonic and hypotonic riboflavin solutions In conclusion, it was observed in the present study that CXL technique with UVA and riboflavin is an effective treatment option for stabilizing the cornea thus arresting the progress of keratoconus in patients with thin corneas (mean thinnest corneal thickness less than 400 μm) as well as remarkably stabilize their visual acuity.

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