

# COMPARATIVE STUDY OF CROSS LINKING VERSUS STROMAL PUNCTURE IN KERATOCONUS

By

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## ABSTRACT

**Background:** Keratoconus is a non-inflammatory condition where the cornea assumes a conical shape because of thinning and protrusion of the corneal stroma. The corneal thinning induces irregular astigmatism, myopia, and corneal protrusion, leading to mild to marked impairment in the quality of vision. Symptoms are highly variable and, in part, depend on the stage of progression of the disorder.

**Objective:** To compare refractive and topographic outcomes between cross-linking (CXL) and corneal stromal puncture in stiffening the cornea and halt the progression of Keratoconus.

**Patients and Methods:** This randomized controlled study included 19 patients (30 eyes) with keratoconus. They were not presenting with any eye disease and had never undergone eye surgery. The total number of subjects meeting the inclusion and exclusion criteria. They were divided into 2 groups, group (A) 9 patient: 15 eyes received cross-linking and group (B) 10 patient: 15 eyes received corneal puncture. All examination, investigation and the procedure were done at El Sayed Galal Hospital and Nour al Hyaa eye Hospital, Cairo.

**Results:** The results showed significant improvement in postoperative value of UCVA, BCVA, than its preoperative value. In addition, a statistically significant decrease of postoperative K1 in both group than its preoperative value while there was a statistically significant increase of K2 postoperative value compared to preoperative value in puncture group, K2 showed significant decrease postoperative in CXL group. There was significant increase in corneal thickness postoperatively compared to preoperative values in puncture group, corneal thickness showed significant decrease postoperatively in CXL group. Comparing both groups, there was significant improvement in postoperative UCVA, postoperative BCVA in puncture group than in CXL group. On the other hand, there was a significant decrease in mean of postoperative corneal thickness in CXL group than puncture group.

**Conclusion:** Overall, the results of this study continue to support the efficacy of CXL in progressive keratoconus and explored new modality of its treatment by anterior stromal puncture (ASP), with an improvement in UCVA, and BCVA 3 months after operation.

**Keywords:** Cross linking, Stromal puncture, Fibrogenesis, Interfibrillary bond, Keratoconus

## INTRODUCTION

Keratoconus is bilateral non-inflammatory, progressive thinning process of the cornea. It is a relatively common disorder of unknown etiology that can involve each layer of the cornea

and often leads to high myopia and astigmatism (*Espandar and Meyer, 2010*).

Keratoconus typically was thought to begin in puberty and progresses until about age 40. Newer imaging modalities, however, have shown that the ectatic condition can occur at a much earlier age

(pre-puberty). It is typically bilateral, but it can be asymmetrical. The overall prevalence of keratoconus has been reported to be between 50 and 230 per 100,000 in the general population, with both sexes equally affected (*Krachmer et al., 2010*).

The etiology of keratoconus may include genetic factors, chromosomal and enzyme abnormalities, and mechanical factors (e.g., eye rubbing) (*Sugar and Macsai, 2012*). The one-third anterior region of the corneal stroma has the greatest cohesive tensile strength, removing it through flap creation might induce corneal biomechanical weakening (*Randleman et al., 2010*).

Patients with keratoconus suffer from varying degrees of disability, including glare, halos, multiple images, ghosting, reduced visual acuity, and intolerance to corrective glasses and contact lenses. The loss of visual function may result in lost productivity, a reduced self-esteem, and difficulties when performing high-skill visual tasks, as keratoconus usually presents in late childhood or adolescence, early diagnosis is very important. The greater the delay of diagnosis, the higher the risk of greater vision loss and of the patient requiring a cornea transplant (*Reeves et al., 2010*).

Once progression is observed early detection and prompt corneal ectasia treatment with corneal cross-linking (CXL), can reduce or stop keratoconus progression and preserve good visual acuity with corrective glasses and/or contact lenses (*Mannis, 2010*).

The most recent treatment is anterior stromal puncture to induce fibrogenesis and interfibrillary bond. The fibrotic

reaction would counteract the fibrinolysis process of ectasia through new collagen production. The result would be an improved biomechanical stability of the ectatic cornea. The deep puncturing spared the visual axis and is performed circumferentially in the paracentral cornea, with denser puncturing at the level of the steepening. The technique is safe and simple with rapid healing, minimal pain, and minimal postoperative scarring. Vertical fibrogenesis is clearly seen on AS-OCT. In addition to a quick recovery, absence of central corneal haze, and a minimal risk for corneal ulcer or epithelial defect, corneal puncturing after previous CXL can be performed in an office setting without the need for expensive equipment (*Jarade et al., 2018*).

**The purpose of this study was to** compare refractive and topographic outcomes between cross-linking and corneal stromal puncture in stiffening the cornea and halt the progression of Keratoconus.

## PATIENTS AND METHODS

This study included 19 patients (30 eyes) with Keratoconus. They were not presenting with any eye disease and had never undergone eye surgery. They were divided into 2 groups, group (A) 9 patient: 15 eyes receive cross-linking and group (B) 10 patient: 15 eyes receive corneal puncture. All examination, investigation and the procedure were done at El Sayed Galal hospital and Nour al hyaa eye hospital, in Cairo.

**Inclusion criteria:** The study included patients with ages from 18 to 40 yrs. according to Amsler-Krumeich classification: Stage I: Eccentric

steepening myopia/astigmatism < 5.00 D (Mean K < 48.0 D). Stage II: myopia/astigmatism > 5.00 D but < 8.00 D (Mean K < 53.0 D). Absence of scarring, minimal apical corneal thickness > 400  $\mu$ m.

**Exclusion criteria:** Patients with corneal thickness of less than 400 microns, herpetic infection, severe corneal scarring or opacification. History of poor epithelial wound healing and severe ocular surface disease (as dry eye) and autoimmune disorders.

All candidates underwent full ophthalmological examination in form of taking good history, slit lamp examination and fundus examination by (90 D lens), visual acuity uncorrected and best corrected by (Log Mar system), measure IOP by (Goldman applanation tonometry), corneal topography by (B&L ORBSCAN 3 Anterior Segment Analyzer).

#### **Operative procedure:**

1. Cross link by Avedro KXL: Under sterile conditions 9 patients (15 eyes) will receive CXL under the standard Dresden protocol (3mW/cm<sup>2</sup> for 30 min, dose 5.4 J/cm<sup>2</sup>). It is performed under topical anesthetic eye drops (Benoxinate). The corneal epithelium is removed (central 8-10mm). 0.1% riboflavin with hydroxypropyl methylcellulose applied to the cornea every 2 minutes for 10 minutes. Then a 9-mm diameter beam of UV-A radiance of 3 m W/cm<sup>2</sup> is irradiated continuously for 30 minutes, resulting in a cumulative dose of 5.4 J/cm<sup>2</sup>. After the procedure, the cornea is rinsed with balanced salt solution, and a drop of 0.5% moxifloxacin is instilled. A silicone hydrogel bandage contact lens is applied, and 0.5% levofloxacin is used 4 times a

day. The bandage contact lens is removed after epithelial healing, and 0.1% fluorometholone 4 times a day is added thereafter. Postoperative medications are tapered over 1 month of the postoperative period.

2. Corneal puncture: 10 patients (15 eyes) is scrubbed with povidone-iodine and draped in a sterile manner. An eyelid speculum is used to hold the eye lids open. The cornea is topically anesthetized. A bent 25-gauge needle is used to vertically puncture the corneal stroma. About 100  $\mu$ m of deep stroma is left unpunctured. The treatment area involves 4.0 to 9.0 mm of the paracentral corneal stroma, at a depth of 350 to 420  $\mu$ m, sparing the central visual axis. It is extended 360 degrees in circumferential rows, with denser puncturing at the level of the corneal steep area or cone. After the procedure, topical antibiotics and steroids are prescribed and slowly tapered over 1 month.

#### **Postoperative assessment:**

The patients followed up for evaluation after surgery as:

- First month after surgery, include refractive and topographic outcome.
- Third month after surgery includes refractive, topographic and anterior segment-OCT (AS-OCT).

#### **Statistical analysis:**

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean  $\pm$  standard deviation (SD). Qualitative data were expressed as frequency and percentage. The following

tests were done: Independent-samples t-test of significance was used when comparing between two means. Paired sample t-test of significance was used when comparing between related samples in parametric data; also Mann Whitney U test: for two-group comparisons and Comparison between two related sample for non-parametric data using Wilcoxon

Rank Sum test in non-parametric data. Chi-square ( $\chi^2$ ) test of significance was used in order to compare proportions between qualitative parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P-value  $<0.05$  was considered significant.

## RESULTS

There was no statistically significant difference between groups according to demographic data. There was statistically significant increase mean of CXL group compared to puncture group according to UCVA. There was statistically significant increase mean of CXL group compared to puncture group according to BCVA.

There was no statistically significant difference between CXL group and puncture group according to K1 and K2. There was statistically significant decrease mean of CXL group compared to puncture group according to postoperative corneal thickness (**Table 1**).

**Table (1): Comparison between CXL group and puncture group according to demographic data, UCVA (log MAR), BCVA (log MAR), K1 & K2 and corneal thickness ( $\mu$ )**

Parameters	Group (A) CXL (n=15)	Group (B) Puncture (n=15)	p-value
<b>Demographic data:</b>			
<b>Sex<sup>#</sup></b>			
Male	6 (40.0%)	7 (46.7%)	>0.05
Female	9 (60.0%)	8 (53.3%)	
<b>Age (years)<sup>¥</sup></b>			
Range	20-42	18-40	>0.05
Mean±SD	29.12±6.91	28.41±7.35	
<b>Laterality<sup>#</sup></b>			
Unilateral	5 (33.3%)	7 (46.7%)	>0.05
Bilateral	10 (66.7%)	8 (53.3%)	
<b>UCVA (Log MAR):<sup>§</sup></b>			
Preoperative	0.80±0.38	0.79±0.25	>0.05
Postoperative	0.75±0.32	0.69±0.27	
<b>BCVA (log MAR):<sup>§</sup></b>			
Preoperative	0.38±0.18	0.39±0.17	>0.05
Postoperative	0.30±0.17	0.20±0.09	
<b>K1:<sup>¥</sup></b>			
Preoperative	45.43±2.99	45.97±2.76	>0.05
Postoperative	44.20±3.04	45.53±2.87	
<b>K2:<sup>¥</sup></b>			
Preoperative	50.09±4.43	49.85±2.81	>0.05
Postoperative	48.62±4.13	50.95±2.73	
<b>Corneal Thickness (<math>\mu</math>):<sup>¥</sup></b>			
Preoperative	465.00±35.77	469.60±23.65	>0.05
Postoperative	383.00±39.42	461.47±21.55	<0.001

Using: ¥t-Independent Sample t-test; #x2: Chi-square test; §z-test: Mann-Whitney test

There was statistically significant improvement of postoperative UCVA, BCVA. In addition, statistically significant decreases of postoperative K1

while a statistically significant increase of K2, while corneal thickness insignificant in puncture group (Table 2).

**Table (2): Comparison between preoperative and postoperative according to UCVA (log MAR), BCVA (log MAR), K1, K2 and corneal thickness in puncture group.**

Group Puncture (n=15)	Preoperative	Postoperative	Mean Diff. ±SD	p- value
UCVA (log MAR) <sup>†</sup>	0.79±0.25	0.69±0.27	-0.1±0.02	0.042
BCVA (log MAR) <sup>†</sup>	0.39±0.17	0.20±0.09	-0.19±0.04	0.003
K1 <sup>‡</sup>	45.97±2.76	45.53±2.87	-0.44±0.08	0.010
K2 <sup>‡</sup>	49.85±2.81	50.95±2.73	1.1±0.21	0.013
Corneal Thickness ( $\mu$ ) <sup>‡</sup>	469.60±23.65	461.47±21.55	-8.13±1.54	>0.05

Using: ‡ Paired Sample t-test; †Wilcoxon test

There was statistically significant decrease mean of postoperative compared to preoperative according to UCVA,

BCVA, K1, K2 and corneal thickness, in CXL group (Table 3).

**Table (3): Comparison between preoperative and postoperative according to UCVA (log MAR), BCVA (log MAR), K1, K2 and corneal thickness in CXL group**

Group CXL (n=15)	Pre	Post	Mean Diff. $\pm$ SD	p-value
UCVA (log MAR) <sup>†</sup>	0.80 $\pm$ 0.38	0.75 $\pm$ 0.32	-0.05 $\pm$ 0.01	0.013
BCVA (log MAR) <sup>†</sup>	0.38 $\pm$ 0.18	0.30 $\pm$ 0.17	-0.08 $\pm$ 0.02	0.011
K1 <sup>‡</sup>	45.43 $\pm$ 2.99	44.20 $\pm$ 3.04	-1.23 $\pm$ 0.23	0.013
K2 <sup>‡</sup>	50.09 $\pm$ 4.43	48.62 $\pm$ 4.13	-1.47 $\pm$ 0.28	0.022
Corneal Thickness ( $\mu$ ) <sup>‡</sup>	465.00 $\pm$ 35.77	383.00 $\pm$ 39.42	-82 $\pm$ 15.58	0.048

Using: ‡ Paired Sample t-test; † Wilcoxon test

There was no statistically significant difference between groups according to K-Max (Table 4).

**Table (4): Comparison between CXL group and puncture group according to Kmax**

	Group (A) CXL (n=9)	Group (B) Puncture (n=10)	p-value
<b>K Max</b>			
<b>Pre-operative</b>			
Range	39.3-51.8	40.5-48.4	>0.05
Mean $\pm$ SD	44.10 $\pm$ 2.21	45.50 $\pm$ 2.01	
<b>Post-operative</b>			
Range	40.6-49.9	41.9-46.6	>0.05
Mean $\pm$ SD	44.22 $\pm$ 1.44	45.57 $\pm$ 1.29	

Using: Independent Sample t-test

## DISCUSSION

The purpose of this study was to compare refractive and topographic outcomes between cross-linking and corneal stromal puncture in stiffening the cornea and halt the progression of keratoconus by restoring the interfibrillar collagen bonds through the induction of collagen fibro genesis. The present study included two groups 19 patient (30 eyes) group (A) 9 patient: included 15 eyes, 3 participants unilateral and 6 participants bilateral received cross-linking (CXL). Group (B) 10 patient: received corneal

puncture and included 15 eyes, 5 participants unilateral and 5 participants bilateral (Puncture). Both groups were comparable in age, sex and laterality.

In current study, within each group, there was significant improvement in postoperative value of UCVA and BCVA than its preoperative value. When we compared there was significant improvement in postoperative UCVA, postoperative BCVA in puncture group than in CXL group.

This study was in harmony with *Wittig-Silva et al. (2014)* at 36 months. In the treatment group, both UCVA and BCVA improved at 36 months.

Moreover, *Jankov et al. (2012)* demonstrated an increase in uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BCVA) compared to controls.

Similarly, *Raiskup-Wolf et al. (2012)* reported their long-term results of 241 eyes from 130 patients with a follow-up for up to 6 years after CXL. This retrospective study confirmed earlier findings of the same group with statistically significant improvement in astigmatism and best corrected visual acuity.

In line with the present study, *Caporossi et al. (2010)* reported a 3.6 line increase in uncorrected visual acuity (UCVA), a 1.66 line improvement in best spectacle corrected visual acuity (BCVA), at 3 months after CXL in a series of 10 eyes in 10 patients with progressive keratoconus.

*Gaster et al. (2013)*, reported on 31 eyes of teenagers who underwent CXL, found significant improvement in UCVA and BCVA, and decreased pachymetry along with no significant complications. They concluded that CXL in young patients is safe, efficacious and should be performed earlier rather than later.

In line with current work, *Jarade et al. (2018)*, five eyes of 3 patients (1 woman, 2 men) with post-LASIK keratectasia had deep stromal puncturing. All patients had improved in uncorrected and best corrected visual acuities, with No

significant postoperative complications occurred in any eyes.

In dis-line with the present study, *Sridhar et al. (2010)* reported a decrease of VA in patients treated with anterior stromal puncture (ASP).

In current study, regarding the puncture group, there were a statistically significance decrease of postoperative value of K1 while a statistically significance increase of K2 than its preoperative value, while both groups were comparable in postoperative K1 and incomparable postoperative K2. In addition, there was no significant difference in postoperative value of K max in both groups.

The current study was line with *Li et al. (2015)* who found, significant decrease in K1 and K2 were demonstrated in CXL group compared with control group.

The current study agreed with *Craig et al. (2014)* who found change in diopter for K1 and K2 in all case the estimated change at 12 months follow up was significant decrease by about 0.75D K1 and 1D K2.

The current study in dis line with *Wittig-Silva et al. (2014)* found that the control group and the treatment group results were different from the current study as in control eyes, K max increased by in treated eyes by CXL, K max flattened.

The current study agreed in K1 value and disagreed in K2, *Kmax with Jarade et al. (2018)*.

In the current study, there was insignificant difference in corneal thickness postoperatively compared to

preoperative values in puncture group, while corneal thicknesses significantly decrease postoperatively in CXL group. On the other hands, there was significant decrease in mean of postoperative corneal thickness in CXL group than puncture group.

The effect of CXL on corneal thickness had so far been less clear. Thinning immediately after CXL has been reported and is thought to be the result of several factors, including treatment-related effects from stromal compaction, postoperative dehydration, and alterations in epithelial healing and distribution. It also may represent a measurement artefact after treatment. Longer-term observations vary from no change in corneal thickness to a decrease at 12 months and an increase at 24 months. Another explanation can be attributed to used device (*Kontadakis et al., 2013*).

Also, the mean change of corneal thickness in CXL group may be related to epithelial remodeling, compactness of collagen fibrils, corneal dehydration, and keratocyte apoptosis (*Choi et al., 2017*).

*Wollensak et al. (2013)* found that there has been an increasing number of case series published reporting the safety and efficacy of CXL treatment in slowing down or halting the progression of keratoconus. Other modalities of treatment were raised. One of them was stromal puncture for inducing fibrogenesis and new interfibrillary bond.

Vitro studies had shown that CXL leads to biochemical and biomechanical changes in both rabbit and human corneal tissue, suggesting that CXL may have a similar effect on the keratoconic cornea and thereby modify the natural course of

the disease (*Spoerl et al., 2010 and 2012*) until now, no study compared between both techniques in keratoconus.

Our results were in agreement with the published literature, since after CXL there was a significant worsening of corneal thickness in the first 3 months with recovery beginning between 3 and 6 months (*Chang & Hersh, 2014 and Choi et al., 2017*).

In accordance with current study, *Madeira et al. (2019)*, reported that, CXL group, there was a decrease in corneal thickness in the first 6 months.

This study was in harmony with *Wittig-Silva et al. (2014)*, who found that, there was a significant reduction in corneal thickness in both groups at 36 months that did not be observed in the treatment group using the manual pachymeter .

Regarding ASP, it was the first study conducted to evaluate the role of anterior stromal puncturing (ASP) in keratoconus. The most important mechanism for ASP is surgery-induced fibrosis, ASP makes the epithelial cells to form a direct contact with the substrate stroma, this can form stable adhesion, serve to strengthen hemidesmosome and fixation filaments. Fibrotic scarring, as a new layer of barrier formation, hinder the aqueous humor from leaking into the sub-epithelium, subsequently, thus corneal big blisters gradually disappear. Corneal nerves exposure could be eliminated with the fibrosis, thereby reducing the cornea perception and pain (*Kenney et al., 2010 and Gregory et al., 2011*).

Similarly, according to *Jarade et al. (2018)*, the anterior stromal puncturing

resulted in localized subepithelial fibrosis that increased the adhesion of the epithelium to the underlying Bowman layer, preventing further erosions and pain which in farther prevent disease progression and increased corneal thickness. However, ASP has fallen out of favor since the introduction of phototherapeutic keratectomy (PTK) in clinical practice, as it can cause scarring and subsequent visual disturbance (*Oikonomakis et al., 2019*).

### CONCLUSION

Overall, the results of this randomized controlled trial of CXL continue to support the efficacy of this treatment of CXL in progressive keratoconus and explored new modality of its treatment by ASP, with an improvement in UCVA, and BCVA 3 months after operation. Furthermore, the risks associated with the procedure seem to be minor relative to the morbidity of advanced disease. The findings of this study suggest that, both ASP and CXL should continue to be considered as a treatment option for patients with progressive keratoconus. And our results favored the ASP as low cost-effective therapeutic modality for keratoconus due to its effects on corneal thickness appeared early than CXL.

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## المقارنة بين الترابط الصليبي للقرنية وبين ثقب القرنية في أمراض القرنية المخروطية

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**خلفية البحث:** القرنية المخروطية هي عبارة عن زيادة تحذب مركز القرنية والمنطقة المحيطة بها ويتغير إلى الشكل المخروطي، مما يسبب اللابؤرية الغير منتظمة التي تشوه الصورة لدى المريض وتسبب انخفاض حدة الابصار. يعد التشخيص المبكر للقرنية المخروطية من اهم الوسائل للحد من تطور المرض وذلك عن طريق فحص تضاريس القرنية (طوبوغرافية).

**الهدف من البحث:** قياس أثر الترابط الصليبي و ثقب القرنية علي مرضي القرنيه المخروطية.

**المرضي وطرق البحث:** شملت الدراسة 30 عين مصابة بالقرنية المخروطية، وتم تقسيمها إلى مجموعتين، تلقت المجموعة الأولى 15 عين العلاج عن طريق الترابط الصليبي، وتلقت المجموعة الثانية 15 عين ثقب القرنية. تمت متابعة المريض للمقارنة بين الإجراءين عن طريق حدة الابصار، رسم تضاريس وسمك القرنية عن طريق استخدام جهاز اورب سكان، قبل وبعد الجراحه في كلا الإجراءين.

**نتائج البحث:** أظهرت النتائج تحسناً ذا دلالة إحصائية في قيمة حدة النظر قبل استخدام النظارة وبعد استخدامها وأيضاً انخفاضاً في K1 بعد العملية الجراحية مقارنة بقيمتها قبل الجراحة، في حين كانت هناك زيادة في قيمة K2 بينما كانت هناك زيادة ذات دلالة إحصائية في سُمك

القرنية بعد العملية مقارنة بقيم ما قبل الجراحة في مجموعة الثقب؛ وقد أظهرت سماكة القرنية انخفاضاً دالاً إحصائياً، بالإضافة إلى انخفاض في قيم كل من K1 و K2 بعد العملية في مجموعة تثبيت القرنية.

**الاستنتاج:** بشكل عام، تستمر نتائج هذه التجربة الخاضعة للرقابة العشوائية في القرنية المخروطية التقدمية في دعم فعالية هذا العلاج (تثبيت القرنية) بعد 3 أشهر من العملية مع استكشاف طريقة جديدة لمعالجتها بواسطة (ثقب القرنية).

**الكلمات الدالة:** الترابط الصليبي للقرنية، البزل اللحمي، التليف الليفي، الرابطة الليفية، القرنية المخروطية.