

Corneal Hysteresis before and after Corneal Collagen Cross Linking for Keratoconus

Magdy Ezz-Eldin Tawakol, Abd El-Magid M. Tag El-Din, Amr Gamal Hassan*

Department of Ophthalmology - Faculty of Medicine, Al Azhar University

*Corresponding author: Amr Gamal Hassan, Mobile: (+20) 01123121500, E-Mail: dr.amr-gamal89@yahoo.com

ABSTRACT

Background: Corneal collagen cross-linking (CXL) has been used as a treatment option in patients with either keratoconus or iatrogenic corneal ectasia after LASIK. The purpose of corneal cross-linking is to stabilize the corneal stroma and to delay the progression of these two pathologic entities.

Objective: The aim of this study is to compare the corneal hysteresis before and after corneal collagen cross-linking using riboflavin and ultraviolet-A light by the epithelial-off technique for treatment of keratoconus.

Patients and methods: This study is a single-centre prospective interventional study. It was conducted between April 2018 to December 2018 and included 30 eyes of 18 patients with a mean age 27.08 years (range from 17- 30). They were 12 female eyes and 18 male eyes; all underwent corneal collagen cross-linking using riboflavin and ultraviolet-A light at Kobry El Kobba Military Specialized Eye Hospital.

Results: All patients were evaluated preoperatively for their uncorrected corrected visual acuity (UCVA), best corrected visual acuity (BCVA), k-reading, spherical equivalent and pachymetry (using Pentacam), and corneal biomechanical properties (using ocular response analyzer (ORA)). The mean age was 27.08 ± 7.86 years, and postoperative follow up was at 1 and 4 months. The mean CH was 7.93 ± 1.79 mmHg preoperatively, 8.27 ± 1.22 mmHg at 1 month, 8.99 ± 1.24 mmHg at 4 months, which show a statistically significant increase in the mean CH pre- and postoperatively, while CRF the other parameter, show a highly statistically significant decrease pre- and postoperatively.

Conclusion: CXL is a promising new treatment modality for keratoconus patients and may affect corneal biomechanics.

Keywords: Corneal Hysteresis, Corneal Collagen cross-linking, Keratoconus, Riboflavin, Ultraviolet-A light.

INTRODUCTION

Keratoconus is a degenerative non-inflammatory corneal disorder. It leads to decreased vision by distorting the anterior corneal surface, and inducing apical thinning, high irregular astigmatism, and central scarring of the cornea. Several modalities such as hard contact lens, intracorneal stromal ring implantation, and penetrating keratoplasty are used to treat keratoconus. All these techniques only correct the refractive error of the cornea with no effect on the progression of keratoconus. The only treatment that is believed to have the ability to stop or decrease the progression of keratoconus is collagen cross-linking (CXL). CXL changes the biomechanical, thermomechanical and morphological properties of the cornea. It increases stiffness and rigidity of the anterior corneal stroma and enhances corneal resistance to proteolytic enzymes by inducing photochemical crosslinking and covalent bindings between individual collagen fibers. CXL halts the progression of keratoconus with a failure rate of approximately 3% and a complication rate of $\leq 1\%$ ⁽¹⁾.

Since CXL alters the corneal shape and structure, it would be helpful to assess resulting changes in quantitative descriptors of the cornea, which can affect the clinical outcome of this procedure. Previous studies have shown that CXL improves visual acuity, average keratometry values, and definable measures of corneal topography regularity. This aimed to evaluate the effect of CXL on corneal biomechanics. Rotating

camera Scheimpflug imagery (Pentacam) provides a multitude of corneal refractive (keratometric), topometric, tomographic, and pachymetric data. In addition, specific anterior-surface irregularity indices have been developed for the grading and classification of keratoconus development, as well as the postoperative assessment. During the last decade, Corneal collagen cross-linking (CXL) technique had gained wide spread successful use in the treatment of ectasias, and many studies had reported that CXL is able to halt progression, stabilize the disease, reduce corneal curvature and improve visual acuity ⁽²⁾.

Corneal collagen cross-linking (CXL) had been shown to stop disease progression in keratoconus over 90% of eyes. This procedure significantly increases the biomechanical strength of the cornea by photochemical crosslinking of individual collagen fibers by utilizing riboflavin and ultraviolet-A light ⁽³⁾.

The ocular response analyzer (ORA) is used to measure in vivo corneal biomechanical properties, which are presented by two parameters corneal hysteresis (CH), and the corneal resistance factor (CRF). It also provides measurements of Goldmann-correlated intraocular pressure (IOPg) and corneal compensated IOP (IOPcc) which are independent of the influence of corneal biomechanical properties ⁽⁴⁾.

Corneal hysteresis (CH) is explained by the viscoelastic structure of the human cornea and its

measurement is an indication of viscous damping in the cornea ⁽⁵⁾.

The ability to repeatedly, accurately, and nondestructively monitor an in vivo biomechanical metric of the cornea that is affected by CXL would allow for better clinical assessment of the treatment and provide a tool to catalyze the further development of the therapy. Clinical studies have quantified the effects of CXL using measures such as refraction, visual acuity, and corneal topography. However, these clinical parameters may not be ideal for monitoring the immediate and progressive biophysical changes associated with CXL. By measuring the mechanical changes caused by CXL, a more fundamental level of data can be obtained to objectively characterize the effects of the therapy. Such a metric may provide a better understanding of the CXL mechanism, allow for improved evaluations of new CXL methods, and potentially offer an approach to more individualized patient care ⁽⁶⁾.

AIM OF THE STUDY

The aim of this study is to compare the corneal hysteresis before and after corneal collagen cross-linking using riboflavin and ultraviolet-A light by the epithelial-off technique for treatment of keratoconus.

PATIENTS AND METHODS

This study is a single-centre prospective interventional study. It was conducted between April 2018 to December 2018 and included 30 eyes of 18 patients with a mean age 27.08 years (range from 17-30). They were 12 female eyes and 18 male eyes; all underwent corneal collagen cross-linking using riboflavin and ultraviolet-A light at Kobry El Kobra Military Specialized Eye Hospital.

Ethical approval and written informed consent:

An approval of the study was obtained from Al-Azhar University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the operation.

Pre-operative evaluation:

Following detailed medical and ophthalmic history, a complete ophthalmic examination was performed including:

- 1) Uncorrected and best corrected visual acuity.
- 2) Anterior segment examination by slit lamp.
- 3) Posterior segment examination using a + 20 diopter lens for indirect ophthalmoscopy, a + 90 diopters lens for slit lamp fundus biomicroscopy.
- 4) IOP measurement by the Goldmann applanation tonometer.

All patients were assessed for corneal curvature and astigmatism by:

Refraction: Objective refraction using the Topcon automated refractometer.

Pentacam: Pentacam was used to assess patients' corneal topography, keratometry and pachymetry. The value of K1, K2 and Kmax were determined together with the size, site and centralization of the corneal cone. The amount of corneal astigmatism was measured. Notation of the astigmatism was done using the difference between the steep and flat corneal meridians. The corneal thickness was measured and the thickest and thinnest locations were determined.

All patients were assessed for corneal biomechanics by:

Ocular response analyzer: Corneal hysteresis (CH) and corneal resistance factor (CRF) were measured for each eye using the Reichert ORA.

Operative Procedure:

Epithelial off technique:

The study included 30 eyes of 18 patients, the preoperative Kmax ranged between 42-51.6 diopters and astigmatism ranged between -1.2 to -8.0 diopters.

All eyes underwent photo-oxidative corneal collagen cross-linking using riboflavin and UV-A light after epithelial debridement of the central 8-9 mm of the cornea. The same CXL machine (UV-X illumination system version 1000; IROC AG) was used in all cases.

This patient underwent riboflavin-UV-A corneal collagen cross-linking (CXL) after removal of the epithelium (epithelium-off).

Anesthesia:

All cases were conducted under topical anesthesia (Benoxinate HCL drops) instilled twice for 2 minutes before the procedure.

Operative details:

After applying the eyelid speculum, an 8-mm diameter marker was used to mark the corneal epithelium in a central circle.

Epithelium was removed in the central 8-9 mm with a blunt metal spatula

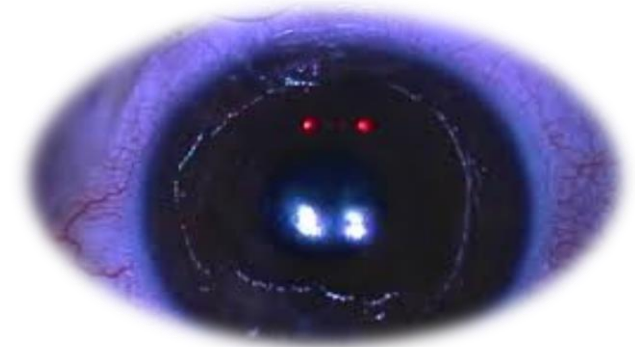


Fig. (1): Epithelium Off.

De-epithelialization was followed by instillation of riboflavin (0.1% solution 10 mg riboflavin-5-phosphate in 10 ml dextran-T-500 20% solution) every 2 minutes for 20-30 minutes until the stroma was completely filled with riboflavin.

After confirming the presence of riboflavin in the corneal tissue and anterior chamber (slit-lamp biomicroscopy), UVA irradiation was applied.

The UVA irradiation was performed using an optical system (UV-X illumination system version - 1000; IROC AG) with a light source consisting of an array of UV diodes (365 nm) in conjunction with a potentiometer to allow regulation of voltage.

Before treatment, an intended 3.0 mW/cm² of surface irradiance (5.4 J/cm² surface dose) was calibrated using a UV light meter at a working distance of 10 cm. Irradiance was performed for 30 minutes.

During treatment, riboflavin solution was applied every 2 minutes to ensure saturation while balanced salt solution (BSS) was used to maintain corneal stromal hydration.

At the end of the procedure, a bandage soft contact lens was kept in place until full corneal re-epithelialization occurred.

III- Postoperative medication:

The postoperative treatment was as follows:

- 1) Topical combined both steroid and antibiotic drop was administrated in all patients 5 times daily for 4 weeks with monitoring intraocular pressure (IOP) then, was tapered to 4 times/day for the next week then 2 times/day for the next 2 weeks.
- 2) Lacrimal substitutes (preservative-free artificial tears) were administered 4 times daily for 4 to 6 weeks.

Postoperative Follow-up:

Patients were followed up postoperatively at 1 and 2 weeks, 1 month, and 3 and 6 months as follows:

- 1) At 1 week, 2 weeks and 1 month postoperative. Patients were examined for their uncorrected visual acuity and best corrected visual acuity, refraction, intraocular pressure, corneal examination, and patients' complaints were all documented
- 2) At the 3rd month and 6th months postoperative. Patients were examined for their uncorrected visual acuity, best corrected visual acuity, refraction, intra ocular pressure, corneal examination, and any complaints.

Patients were investigated then as follows:

- i. Pentacam: to assess the patient's topography, keratometry, corneal astigmatism and pachymetry.
- ii. Ocular response analyzer: to measure corneal hysteresis and corneal resistance factor.

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±standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:
 P-value <0.05 was considered significant.
 P-value <0.001 was considered as highly significant.
 P-value >0.05 was considered insignificant.

RESULTS

Our study was a single-centre prospective interventional clinical study:

Thirty (30) eyes of eighteen (18) patients were included in the study. Their demographic data are in table 1.

Table (1): Demographic characteristics of the patients.

		Patients group
		No. = 30
Age	Mean ± SD	27.08 ± 7.86
	Range	17 – 30
Sex	Male	18 (60.0%)
	Female	12 (40.0%)

Visual and refractive outcomes:

Uncorrected visual acuity (Fig. 2 and tables 2 and 3):

There is a highly statistically significant improvement in the mean UCVA postoperatively compared with that of preoperative.

Best spectacle corrected visual acuity (Fig. 2 and tables 2 and 3):

There is a statistically significant improvement in the mean BSCVA postoperatively compared with that of preoperative.

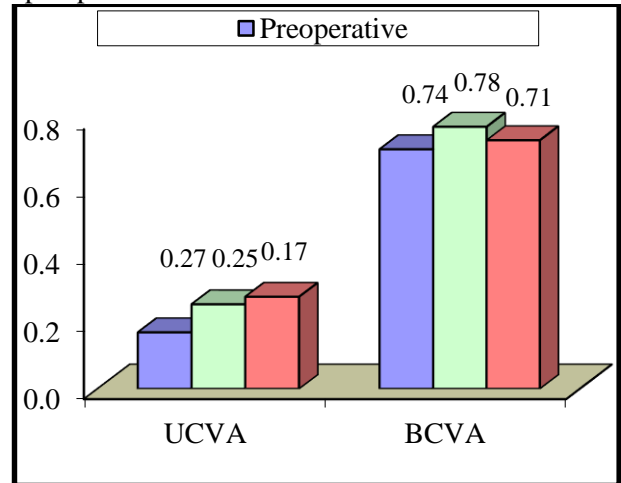


Fig. (2): Comparing visual acuity (UCVA and BCVA) preoperative and 1 month 4 month postoperative.

Spherical equivalent (S.E.) (Fig. 3 and tables 2 and 3):

There is a highly statistically significant difference between the result of S.E. before and 1 month and 4 month postoperatively.

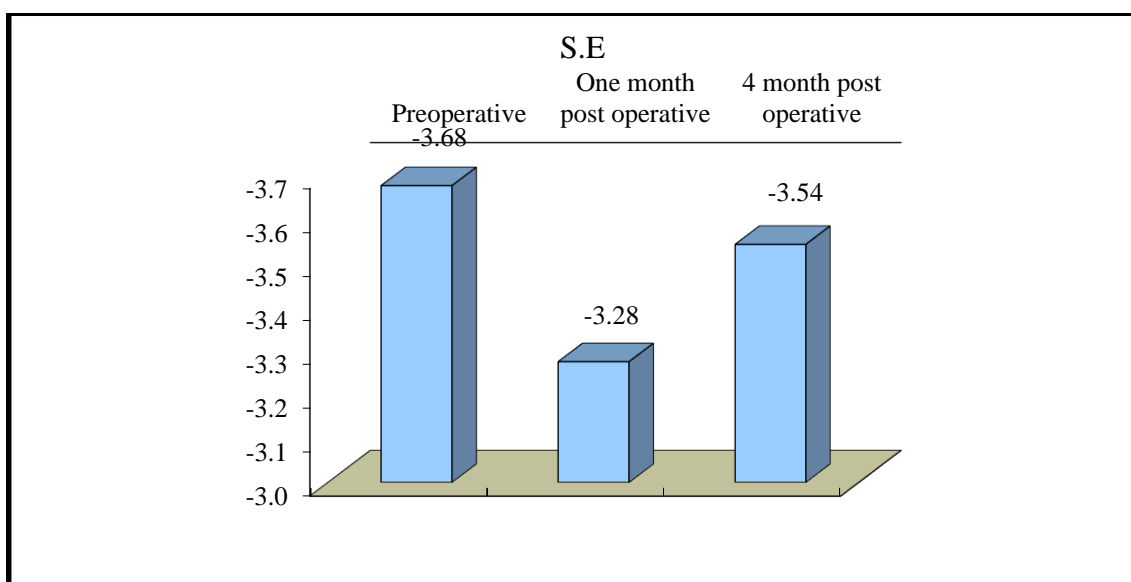


Fig. (3): Comparing between the mean spherical equivalent preoperative and 1 month and 4 month postoperatively.

Table (2): UCVA and BCVA and S.E. before and after surgery.

		Preoperative	One month	4 month	P-value
		No. = 30	No. = 30	No. = 30	
UCVA	Mean ± SD	0.17 ± 0.12	0.25 ± 0.16	0.27 ± 0.14	0.001
BCVA	Mean ± SD	0.71 ± 0.15	0.78 ± 0.16	0.74 ± 0.17	>0.05
S.E	Mean ± SD	-3.68 ± 1.11	-3.28 ± 1.14	-3.54 ± 1.24	0.001

Table (3): Post Hoc analysis by Bonferroni.

	Post Hoc analysis by Bonferroni		
	P1	P2	P3
UCVA	0.001	0.001	0.015
S.E	0.001	>0.05	>0.05

P1: Preoperative Vs 1 month month

P2: Preoperative Vs 4 month

P3: 1 month Vs 4 month

Table (4): Comparative study between preoperative and postoperative mean K-readings at 1 and 4 months.

		Preoperative	One month postoperative	4 month postoperative	P-value
		No. = 30	No. = 30	No. = 30	
K1	Mean ± SD	47.45 ± 4.41	48.74 ± 4.90	47.92 ± 4.77	>0.05
K2	Mean ± SD	50.78 ± 4.34	51.86 ± 4.94	51.93 ± 4.53	>0.05
K-max	Mean ± SD	57.18 ± 5.61	57.19 ± 6.45	57.68 ± 6.33	>0.05

Table (5): Comparative study between corneal biomechanics (CH, CRF and corneal thick) pre and 1 month and 4 month.

		Preoperative	One month postoperative	4 month postoperative	Test value•	P-value
		No. = 30	No. = 30	No. = 30		
CH	Mean ± SD	7.93 ± 1.79	8.27 ± 1.22	8.99 ± 1.24	5.265	<0.02
CRF	Mean ± SD	8.32 ± 2.72	6.81 ± 1.41	7.29 ± 1.36	10.201	<0.02
Thickness	Mean ± SD	476.53 ± 51.46	463.50 ± 50.36	465.03 ± 38.26	3.639	>0.05

Table (6): Post Hoc analysis by Bonferroni.

	Post Hoc analysis by Bonferroni		
	P1	P2	P3
CH	>0.05	>0.05	0.006
CRF	0.004	0.018	>0.05

P1: Preoperative Vs 1 month

P2: Preoperative Vs 4 month P3: 1month Vs 4 month

Table (5 and 6) show a comparison at the results of CH, CRF and corneal thickness at preoperative, one month postoperatively and 4 month postoperatively.

Table (7): Show the relationship between UCVA, BCVA, S.E. K2, K-max, thickness with both CH and CRF preoperatively.

	Preoperative			
	CH		CRF	
	r	P-value	r	P-value
UCVA	0.136	>0.05	0.345	>0.05
BCVA	-0.417*	0.022	0.355	>0.05
S.E	-0.320	>0.05	0.243	>0.05
K1	0.172	>0.05	-0.294	>0.05
K2	0.441*	0.015	-0.221	>0.05
K-max	0.205	>0.05	-0.370*	0.044
Thickness	-0.383*	0.037	0.683**	0.000

Table (8): Show the relationship between UCVA, BCVA, S.E. k1, K2, K-max, thickness with both CH and CRF 1 month postoperatively.

	Postoperative (1month)			
	CH		CRF	
	r	P-value	r	P-value
UCVA	0.145	>0.05	-0.013	>0.05
BCVA	0.083	>0.05	-0.026	>0.05
S.E	-0.017	>0.05	-0.160	>0.05
K1	-0.219	>0.05	-0.427*	0.019
K2	-0.217	>0.05	-0.379*	0.039
K-max	-0.193	>0.05	-0.373*	0.042
Thickness	0.282	>0.05	0.448*	0.013

Table (9): Show the relationship between UCVA, BCVA, S.E. K-readings, corneal thickness with both CH and CRF 4 month postoperatively.

	Postoperative (4 month)			
	CH		CRF	
	r	P-value	r	P-value
UCVA	-0.117	>0.05	0.127	>0.05
BCVA	-0.492**	0.006	-0.302	>0.05
S.E	-0.457*	0.011	-0.238	>0.05
K1	-0.179	>0.05	-0.247	>0.05
K2	0.019	>0.05	-0.133	>0.05
Kmax	0.107	>0.05	-0.124	>0.05
Thickness	0.332	0.073	0.618**	0.000

DISCUSSION

In recent years, it has been reported that CXL therapy slow, stops and even reverses keratocones. In

vitro studies have demonstrated that the treatment increase the number of crosslink in the stroma and thus enhances the biomechanical resistance of the cornea. In a 2003 in vitro study conducted with a strip extensometer, **Wollensak et al.** ⁽⁷⁾ experimentally demonstrated that young's modules, which indicate the biomechanical rigidity of the cornea, increased 4.5 times in the human cornea and 1.8 times in the porcine cornea after CXL. However this method is not suitable for clinical use because it is done with stripped corneal tissue.

Devices that assess corneal biomechanics in vivo are the ORA, the corvis tonometer and applanation resonance technology. The ORA is most commonly used in the clinic for evaluating corneal biomechanics, and there are many studies based on ORA results after various ocular pathologies and eye surgeries. ORA studies performed in keratoconus have reported lower CH and CRF parameters compared with normal eyes. Following corneal transplantation in eyes with advanced keratoconus, CH and CRF were increased but were found to be lower compared to normal eyes⁽⁸⁾.

In our study; patients showed a highly statistically significant improvement of the UCVA postoperatively. The mean preoperative UCVA was 0.17 ± 0.12 , while the mean postoperative UCVA at the 1st visit (1 month) was 0.25 ± 0.16 , and the mean postoperative UCVA at the last visit (4 month) was 0.27 ± 0.14 , improving by a mean of +0.8, +1.00 Snellen lines respectively.

Also improvement in the BCVA was significant, where the mean preoperative BCVA was 0.71 ± 0.15 , while the mean postoperative BCVA at 1 month visit was 0.78 ± 0.16 , and the mean postoperative BCVA at 4th month visit was 0.74 ± 0.17 , improving by a mean of +0.7, +0.3 Snellen lines respectively.

The above results are comparable with the results of the study conducted by **Caporossi et al.** ⁽⁹⁾ (study included 44 eyes) which stated that the mean UCVA improved by a mean of +2.41 Snellen lines after 12 months, +2.75 Snellen lines after 24 months, where **Caporossi et al.** ⁽⁹⁾ stated that BCVA improved by a mean of +1.34, +1.93 Snellen lines 12 month, 24 month postoperatively, respectively.

In our study, the improved UCVA recorded during the follow up was partially explained by the sphere and spherical equivalent reduction.

Topographic analysis done for our patients preoperatively revealed a mean K-max of 57.18 ± 5.61 , the mean postoperative at 1 month, 4 month postoperative was 57.19 ± 6.45 , 57.68 ± 6.33 , respectively, which mean that there was no significant difference of the mean K-max pre and postoperative.

Kymionis et al. ⁽¹⁰⁾ (study on 14 eyes maximum follow up 12 months) had flattening of the mean keratometry reading.

One of the most important criteria to evaluate the effects of CXL in our study was to evaluate its effect on the corneal thickness, thus the patient's pachymetry was recorded throughout the study using the Oculus Pentacam.

The mean preoperative pachymetry at its thinnest location was 476 ± 51.46 , after 1 month, the mean pachymetry (thinnest) was 463.50 ± 50.36 , and 4 month postoperative the mean pachymetry (thinnest) was 465.03 ± 38.26 .

Our results revealed a statistically significant decrease in the mean corneal thickness pre, 1 month, 4 months postoperatively.

Similar results were found by **Vinciguerra et al.** ⁽¹¹⁾ that there was a significant decrease in the corneal thickness after 12 month of follow up.

Because CXL supposed to halt the progression of keratoconus by increasing the stiffness of the cornea, we measured CH and CRF using ORA expecting to observe changes after treatment.

In our study; the mean preoperative corneal hysteresis (CH) was 7.93 ± 1.79 mmHg, while the mean CH postoperatively at 1 and 4 month was 8.27 ± 1.22 , 8.99 ± 1.24 respectively, which mean that, there was a statistically significant increase in the mean CH pre and 1 month and 4 month postoperatively.

Also the mean CRF preoperative was 8.32 ± 2.72 mmHg, while the mean CRF at 1 and 4 month postoperative was 6.81 ± 1.41 and 7.29 ± 1.36 mmHg respectively, which mean that there was a highly statistically significant decrease in the mean CRF pre and postoperatively.

ORA studies performed in eyes with keratoconus following CXL report different results. **Küçümen et al.** ⁽⁸⁾ (study included 35 eyes with a mean follow up 20.2 ± 14 months) reported that the mean CH was increased in the early and late post CXL periods compared to the preoperative level, but the difference was not statistically significant, and also reported that the mean CRF was found to be significantly increased in the early post CXL period and also increased in the late post CXL period compared with preoperative period, but the changes were not statistically significant.

Çağıl et al. ⁽¹²⁾ also found a non-significant increase in CH or CRF values at postoperative 1 and 6 months. **Vinciguerra et al.** ⁽¹¹⁾ reported increases in CH and CRF values at postoperative 1 month. However, they found no significant difference at postoperative 6 and 12 months compared to the preoperative values. **Greenstein et al.** ⁽¹³⁾, also found a significant increase in CRF at 1 and 3 months but found no significant difference at 1 year.

CXL aimed to increase the rigidity and resistance of the cornea. An increase in the values of parameters that measure corneal biomechanics is expected after CXL. Therefore, theoretically, a statistically significant increase would be expected after CXL in the biomechanical indicators assessed by the

ORA device, especially CH and CRF values. In our study we observed that increase of the one parameters, which is corneal hysteresis while decrease in the other parameter, which is corneal resistance factor.

The fact that our study differs in some results from the other studies may be due to various reasons. The first of them is the low number of patients, which is the main limitation of our study and the short follow up period postoperatively.

Another reason may be that each eye with keratoconus has different configuration, pachymetric, topographic, and therefore biomechanical properties. It is argued that because the cornea is not homogeneous, the ORA device may not be technologically sufficient for measurements. Other studies have similarly addressed the possibility of optical irregularities in ectatic corneas obscuring actual biomechanical changes by altering ORA signals. With time, the development of more precise versions and/or new devices may yield more meaningful results ⁽¹¹⁾.

CONCLUSION

CXL is a promising new treatment modality for keratoconus patients and may affect corneal biomechanics. Larger patient series and more advance technologies are needed to fully understand corneal biomechanics and to quantitatively and precisely assess them. Devices that can accurately measure these changes and are suitable for clinical use have not yet been developed, including the ORA. To understand the mechanism of action of this therapy on the cornea, there is a need for multicenter, randomized, prospective studies including large patient population with long postoperative follow up periods, which will provide more statistically valuable results.

RECOMMENDATION

CXL is recommended as a successful, safe and in expensive procedure for patients with corneal ectasia, that help to delay, if not prevent the need for corneal transplantation later on and to achieve a good visual quality for these patients.

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