Corneal Biomechanical Properties before and after Collagen Cross Linking in Keratoconus

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Abstract

Aim of Study: The aim of this study is to analyze the changes in the biomechanical properties of the cornea after collagen cross linking in eyes with progressive keratoconus through the assessment of corneal hysteresis and corneal resistance factor before and after the procedure.

Methods: Thirty eyes of 22 patients with mean age 24.56±3.79 years with progressive keratoconus were treated with UV-A-ribbon Collagen Cross Linking (CXL) and assessed with the Ocular Response Analyzer (ORA) that measured Corneal Hysteresis (CH), Corneal Resistance Factor (CRF), Goldmann-correlated intraocular pressure (IOPg), and corneal compensated intraocular pressure (IOPcc). Patients were assessed with ORA preoperatively, at 1 and 6 months after treatment. Postoperative measurements at each visit were compared with preoperative values. Demarcation line and corneal haze were also assessed after cross linking procedure.

Results: CH was decreased after cross linking treatment, with the difference not statistically significant at 1 and 6 months p-value=0.61 and 0.198 respectively. CRF was elevated at 1 and 6 months after CXL and was statistically significant at 6 months (p=0.005).

Conclusion: After collagen cross linking no significant change in corneal hysteresis occurred at 1 and 6 months. Increase in corneal resistant factors occurred and was statistically significant at 6 months.

Key Words: Keratoconus – Collagen cross linking – Corneal hysteresis – Corneal resistance factor – Ocular response analyzer.

Introduction

KERATOCONUS is a progressive, non inflammatory dystrophy of the cornea of unknown pathogenesis, characterized by a number of histopathologic abnormalities, which lead to para axial stromal thinning and weakening that leads to corneal surface distortion. Visual loss occurs primarily from irreg-

ular astigmatism and myopia, and secondarily from corneal scarring. Protrusion usually but not exclusively affects the axial and inferonasal cornea [1].

In early stages of keratoconus corneal Collagen Cross Linking (CXL) is a safe and effective procedure used to increase the rigidity of the cornea by inducing additional cross links within or between collagen fibers using UVA light and a photomediator, riboflavin (vitamin B2), with the goal of slowing down, possibly stabilizing, and even perhaps reversing, the progression of corneal ectasia in patients with keratoconus [1,2].

Collagen cross linking in keratoconus can be performed in combination with surface ablation to correct residual refractive errors. A combination of topography-guided custom ablation and CXL was found to improve patients' visual, refractive, and topography outcomes and halted the progression of keratectasia [3].

Ocular Response Analyzer (ORA) is a device able to provide an in vivo dynamic measurement of corneal viscoelastic behavior. It is a non contact tonometer which allows measurement of intraocular pressure as well as other parameters called corneal hysteresis and corneal resistance factor. ORA is based on dynamic bidirectional applanation. It releases a precisely metered air pulse causing the cornea to move inwards (inward applanation), and then to the past applanation phase where it becomes slightly concave. Milliseconds after the air puff shuts off the pressure decreases. During this phase when the cornea is trying to regain its normal shape, it passes through an applanation phase (outward applanation). Theoretically, these two pressures should be the same but are actually different. This difference is described as the dynamic corneal response which is said to be the

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Corneal biomechanics refers to the mechanical properties of the corneal tissue due to its viscoelastic properties. This difference between the outward and inward pressures is termed corneal hysteresis [4,5].

Material and Methods

All patients received a thorough explanation of the study design and aims. Study participants gave informed consent before initiation of any study-related procedures, and the study was conducted in compliance with informed consent regulations.

This is a prospective, interventional, nonrandomized and non-controlled study. It was carried out between 2011 to 2013 in Rowaad vision correcting center. The protocol was revised and approved by our Ophthalmology Department ethical committee.

Inclusion criteria:
Clear ocular media, corneal stromal thickness in the thinnest point at least 400 microns and their age more than 18 years either males or females, during the determined time period of the study.

Exclusion criteria:
History of herptic keratitis, any corneal infection, corneal stromal thickness less than 400 microns at any point, severe dry eye, diabetes and concomitant autoimmune diseases, any previous ocular surgery, pregnant or lactating females and patients with central or paracentral corneal opacities.

Preoperative evaluation:
- Full medical history (duration of diabetes, controlled or not), other systemic diseases.
- All eyes were subjected to examination of Best Corrected Visual Acuity (BCVA) using snellen’s chart.
- Slit lamp examination of anterior segment to exclude corneal abnormalities and significant cataract.
- Intraocular pressure measurement using Goldman applanation tonometry.
- Fundus examination was done using indirect ophthalmology.
- Pentacam tomographic evaluation using (Allegro oculoyzer, WaveLight AG, Erlangen, Germany).
- ORA measurements using (Reichert Ophthalmic Instruments, New York, USA).

Surgical technique:
Riboflavin 0.1% was prepared by diluting vitamin B2-riboflavin-5-phosphate 0.5% with dextran T500 to achieve a 0.1% riboflavin solution. The solution was protected from light and used within 12 hours.

Topical anesthesia was applied before the procedure using benoxinate eye drops. The corneal epithelium was mechanically removed over an area of 8mm in diameter using a blunt instrument (Hockey knife), and riboflavin 0.1% solution was instilled repeatedly for approximately 30 minutes. Penetration of the cornea and presence of riboflavin in the anterior chamber (ribo-flavin shielding) was monitored with slit-lamp examination using blue filter. UVA irradiation was performed using an optical system (Kohler illumination) with a light source consisting of an array of 7 UV diodes (365nm; Nichia, Nuernberg, Germany) with a potentiometer in series to allow for regulation of voltage. Before treatment, intended irradiance of 3mW/cm² surface irradiance (5.4 J/cm² surface dose) was calibrated using a UVA meter (LaserMate-Q; LASER 2000, Wessling, Germany) at a working distance of 1cm. Irradiance was performed for 30 minutes by using 3mW/cm², corresponding to a dose of 5.4J/cm². During treatment, riboflavin solution was applied every 3 minutes to saturate the cornea with riboflavin. After the treatment, a bandage contact lens was applied until complete regeneration of the corneal epithelium which was usually achieved completely after 3 days. Postoperatively patients were given topical antibiotic with artificial tear substitutes.

Postoperative care:
After removal of contact lens the patient is prescribed topical combination of steroid and antibiotic six times daily, with a gradual decrease over the following 6 weeks together with topical lubricant substitute. Patients were examined every 2 days until complete epithelialization then at 1 and 6 months using slit lamp examination, ocular response analyzer and anterior segment OCT using RTVue-100 Fourier-Domain OCT from Optovue.

Results

Demographic data analysis:
The study included 30 eyes of 22 patients, 14 of which were males (46.3%) and 16 were females (53.3%) as shown in Fig. (1), with mean age of 24.56±3.79 years, ranging from 19 to 35 years.
Corneal biomechanics (ORA results):

- **Corneal hysteresis and corneal resistance factor:**
  
  The mean preoperative Corneal Hysteresis (CH) was 8.04±0.63 mmHg ranging from 6.8 to 9.4 mmHg and Corneal Resistance Factor (CRF) was 6.98±0.92 mmHg ranging from 5 to 9.4 mmHg. Mean CH values insignificantly decreased after 1 and 6 months to be 8.00±0.64 mmHg and 7.9±0.636 mmHg, respectively where \( p \)-value is 0.61 and 0.198 respectively. Mean CRF was 7.4±0.95 mmHg and 7.47±1.0 mmHg at 1 and 6 months respectively after CXL thus showing statistically significant increase where \( p \)-value was 0.007 and 0.005 respectively. [As shown in (Table 1) and Fig. (3)].

- **Goldmann-correlated intraocular pressure (IOPg) and corneal compensated intraocular pressure (IOPcc).**

  Mean preoperative IOPg was 11.22 mmHg±2.74 mmHg ranging from 6 to 17.5 mmHg. It significantly increased after 1 month from the collagen cross linking to be 12.06±2.53 mmHg ranging from 9 to 18 mmHg with \( p \)-value 0.004, while after 6 months there was insignificant increase in the IOPg to be 11.36±2.31 mmHg ranging from 8 to 18.7 mmHg with \( p \)-value 0.745.

  Mean preoperative IOPcc was 14.52 mmHg±2.54 mmHg ranging from 9.6 to 21 mmHg. It increased insignificantly after 1 month from the collagen cross linking to be 14.96±2.01 mmHg ranging from 12 to 20 mmHg with \( p \)-value 0.187 as well as after 6 months to be 14.76 mmHg±2.01 mmHg ranging from 10.9 to 20 mmHg with \( p \)-value 0.431, as shown in [Fig. (4) and (Table 2)].

Demarcation line using anterior segment OCT:

Demarcation line after CXL was found at 1 month in 26 eyes (86.66%) and absent in 4 eyes (13.34%) and disappears at 6 months in all eyes as shown in Fig. (7). Mean level of the demarcation line in the cornea was 303.98±10.37 μm from the surface of corneal epithelium ranging from 280 to 320 μm as shown in Fig. (6). Females were found to have shallower demarcation line than males as shown in (Table 3).

Complications:

**Corneal haze:**

Temporary corneal haze was found in all eyes after CXL with different degrees detected by slit-lamp biomicroscopy gradually disappears after 1 month with steroid treatment as shown in Fig. (6). All corneas were clear at 6 months.

Delayed re-epithelialization no delayed re-epithelialization occurred in any of the patients.

**Night glare and haloes:** 90% of patients complained of night glare and haloes, but only in the first 3 months.

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<th>Table (1): Comparing preoperative and postoperative mean CH and CRF readings at 1 and 6 months.</th>
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<td>Mean CH</td>
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<th>Table (2): Comparing changes in IOPcc and IOPg through the study and its significance.</th>
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<th>Table (3): Mean demarcation line depth in males and females.</th>
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<th>Table (4): Severity and sex distribution of haze at 1 month after CXL.</th>
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**Haze:**

- Mild: 14
- Moderate: 1
- Severe: 1

Total: 23

30
Fig. (1): Male to female percentage in our study.

Fig. (2): Preoperative and 1 and 6 months postoperative changes in mean CH and CRF.

Fig. (3): Changes in CH and CRF before (a) and after CXL at 1 (b) and 6 (c) months.

Fig. (4): Graph comparing both preoperative and 1 and 6 months postoperative changes in mean IOPcc and IOPg.

Fig. (5): Graph showing percentage of demarcation line at 1 month after CXL.

Fig. (6): An anterior segment OCT scan of at 1 month after CXL showing demarcation line at 320um.

Fig. (7): Slit-lamp photo showing subepithelial reticular appearance of corneal haze 1 month after CXL.
Discussion

The mean preoperative Corneal Hysteresis (CH) was 8.04±0.63mmHg. Mean CH values were decreased at 1 and 6 months after CXL (mean CH was 8.00±0.64mmHg at 1 month and was 7.90±0.63 at 6 months which was statistically insignificant. Mean preoperative CRF was 6.98±0.92mmHg. There was a statistically significant increase in the CRF in the 1st month and at the 6th month where CRF was (7.4±0.95mmHg and 7.47±1.0 respectively).

CH is the result of the viscous damping within corneal tissues that is created by the viscosity of Glycosaminoglycans (GAGs) and proteoglycans [6]. It was assumed that the insignificant decrease in CH after CXL may be due to stromal edema, matrix reorganization and reduction in glycosaminoglycans synthesis resulting from stromal cell death [7].

CRF is a measure of overall resistance of cornea caused by ground substance (GAGs), proteoglycans and collagen fibrils which undergo stiffening after CXL explaining the significant increase in CRF after CXL at 6 months.

Goldich et al., conducted a study on 10 eyes. They found statistically insignificant decrease in CH after CXL but CRF remain stable. Their preoperative mean of CH and CRF were 8.44 and 7.15mmHg respectively. At 1 month the values were 8.22 and 7.91mmHg and at 6 months were 8.14 and 7.16mmHg respectively [8].

Sedaghat et al., conducted study on 56 eyes they found statistically non-significant increase in CH and CRF at 6 months after CXL. Mean preoperative values was 7.9±1.5mmHg and 7.3±1.4 mmHg and at 6 months values were 8.2±1.5mmHg and 7.59±1.5mmHg respectively [5].

Gikika et al., conducted a study on 30 eyes. They found non-significant increase in CH and CRF at 6 months. Mean preoperative CH was 8.2±1.4mmHg and CRF was 7.4±2.3mmHg and at 6 months mean CH was 8.7±1.1mmHg and mean CRF was 7.6±1.6mmHg [9].

In our study we found statistically significant increase in IOPg and non-statistically significant increase in IOPcc at 1 month and both showed non-statistically significant increase at 6 months. This is can be explained by the change in the corneal rigidity which is correlated with the increase in the CRF which seems to overweigh the effect of corneal thinning on the IOP measurement.

Our results were similar to a study conducted by Goldish et al., on 10 keratoconic eyes who found statistically significant increase in IOPg and IOPcc at 1 month after CXL and non-statistically significant increase at 6 months (mean preoperative values was 10.2±1.63mmHg, 13.6±2.06mmHg respectively at 1 month was 13.2±3.55 mmHg and 16.5±3.57mmHg at 6 months values was 11.2±2.89mmHg and 14.7±2.087mmHg).

On contrary of our results a study conducted by Sedaghat et al., who found non-significant decrease in IOPg and statistically significant decrease in IOPcc at 6 months (mean preoperative IOPg 10.47±3.0mmHg and at 6 months 10.07±3.0 mmHg mean preoperative IOPcc was 13.98±2.9 mmHg and at 6 months 13.14±2.8mmHg).

Demarcation line was detected at 1 months after collagen cross linking in 26 eyes (86.66%) using anterior segment OCT and disappeared at 6 months follow-up. Mean demarcation line depth at 1 month was 303.98±10.37um.

Demarcation line is a whitish opaque line representing a transition zone between cross linked anterior stroma (showing edema and hyporeflectivity) and non-crossed posterior stroma which is normal. It represents activation of keratocytes which is followed by repopulation of keratocytes and new collagen synthesis [10].

Doors et al., conducted a study on 29 eyes. Demarcation line was visible in 28 eyes at 1 month after CXL and disappeared in all included eyes at 6 months postoperatively. Mean demarcation line depth was 313±61um. In 75% of examined eyes demarcation line was easily to be detected but in 25% of eyes it was difficult to be detected and recorded as visible [11].

Kyminios et al., did a study on 18 keratoconic eyes. Mean demarcation line depth at 1 month after CXL using anterior segment OCT was 300±41.56um [12].

In our study we found that females have a shallower demarcation line depth compared to males. The same results were observed by Yam et al., in 2012. We found positive statistically insignificant correlation between demarcation line depth and preoperative Kmax on contrary to Yam et al., who found negative correlation (the more the steepening the shallower the demarcation line) [13].

One of most obvious complication we found after CXL is corneal haze which was detected in all eyes after CXL in different degrees. This haze
was temporary and gradually disappears after 1 month with steroid treatment. Corneal haze had subepithelial reticulated appearance.

After CXL, there is increase in collagen fibrils diameters and increase in spacing is detected which may play a role in decreasing corneal transparency. Also repopulation of keratocytes and changes in its cellular components after CXL is detected, all may play a role in haze development (Wollensak et al., 2004).

References