# Corneal Endothelial Cell Changes After Corneal Cross Linking In Keratoconus

May Hassan Mohamed, Mohamed Wahby El Kateb, Shahira Mahmoud Rashad, Abd El Hamid El Houfy

[a]Department of Ophthalmology, Faculty of Medicine at Alexandria University, Egypt

Correspondence to May Hassan Mohamed, MD, Alexandria

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**Aim:** To assess the corneal endothelial cell changes after accelerated corneal cross linking (CXL) in keratoconus patients.

**Methods:** 60 eyes of 60 patients who undergone accelerated CXL for progressive keratoconus were enrolled. The preoperative and three months postoperative endothelial cell density (ECD) and coefficient of variation (CV)- measured by specular microscopy- together with the central corneal thickness (CCT) -measured by Pentacam -were compared. Intraoperative CCT was also measured intraoperatively using the built-in pachymeter before UV irradiation. We divided the patients into two groups according to intraoperative CCT: below or above 386 µm.

**Results:** The mean preoperative ECD was 2604.25 ± 222.54 cell/mm2 that decreased to 2584.53 ± 262.55 cell/mm2 postoperatively. The mean CV increased from 39.72 ± 3.84% preoperatively to 40.08 ± 4.17 post operatively. The change in ECD and CV was not statistically significant (p=0.14& 0.16, respectively). There was a statistically significant reduction in the CCT from 492.88 ± 35.08 µm to 482.60 ±37.68 µm postoperatively (p=0.001). According to intraoperative CCT, there was no significant difference between the two groups (below or above 386 µm) regarding the ECD, CV, and percentage of endothelial cell loss (p=0.1&0.9&0.6, respectively).

**Conclusions:** Accelerated crosslinking is safe in terms of corneal endothelial cell changes. The intraoperative reduction in CCT seems to not affect the endothelial cells significantly even in those with thickness less than 386 µm.

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## Introduction

Keratoconus is a progressive thinning disorder of the cornea that results in vision loss because of the induced irregular astigmatism.[1] The prevalence of keratoconus ranges from 0.5 to 2.3 per 1000 population.[2] Corneal collagen cross linking (CXL) was recently introduced as a treatment modality that strengthens the cornea and halts the progression of the disease.[3] The procedure involves the installation of riboflavin that acts as a photosensitizer followed by irradiation of the cornea with ultraviolet A (UVA). The interaction between the riboflavin and UVA results in the production of reactive oxygen species, which induces the formation of covalent bonds between the collagen fibrils.[4] CXL is indicated for patients with progressive keratoconus.[5] Early introduction of CXL can delay or avoid the need for keratoplasty. However, CXL is not a procedure without complications. The UVA used during the procedure can damage the corneal endothelium with subsequent corneal edema that may require keratoplasty in rare cases.[6] CXL is performed if the corneal thickness is more than 400 microns after de-epithelization to prevent endothelial toxicity.[7, 8] Some studies have shown that the corneal thickness is reduced significantly during the procedure due to dehydration of the cornea that may result in endothelial cell damage.[9] The present study aimed to assess the corneal endothelial changes after corneal cross linking in keratoconus and the intraoperative change in corneal thickness and its effect on the corneal endothelium.

## Patients & Methods

This prospective, non-randomized study was conducted in a private care center in Alexandria, Egypt. The study was approved by the Institutional Ethics Committee and followed the tenets of the Declaration of Helsinki. All patients signed a written informed consent. The study included 60 eyes of 60 patients with documented keratoconus progression. Keratoconus progression was diagnosed according to the criteria established by the global consensus on keratoconus and ectatic diseases.[10] The exclusion criteria were patients with central corneal opacities, history of herpetic keratitis, autoimmune disease, severe dry eye, acute hydrops, vernal keratoconjunctivitis, or thinnest corneal pachymetry less than 450 µm Preoperatively, all patients underwent thorough ophthalmic examination including uncorrected and best-corrected visual acuity (UCVA and BCVA), slit-lamp GmbH, Wetzlar, Germany), and specular

examination, dilated fundus examination, imaging with the Oculus Pentacam (Oculus Optikgerate microscopy using the Tomey EM-3000™ (CBD/Tomey, Phoenix, aZ, USA).

Postoperatively, the patients were examined on days one and seven and one and three months. The Pentacam and specular microscopy examinations were repeated at three months.

One surgeon conducted all the CXL in the operating room. After installation of topical anesthesia, a lid speculum is inserted, and the epithelium in the central 8mm is removed with mechanical scraping. The riboflavin solution (Ribolex® Sterile Isotonic Solution 3mL) is then applied every 5 minutes for 30 minutes. The intraoperative CCT was measured immediately before UVA irradiation by the WaveLight® EX500 built-in pachymetry (Alcon Laboratories, Fort Worth, TX, USA). The UVA was applied for 5 minutes (irradiation level 18 MW/ cm2, 5.4 J/cm2 cumulative irradiation dose). At the end of the procedure, antibiotic drops are installed then a bandage contact lens is placed. Postoperatively, the patients are prescribed topical antibiotics four times per day for one week and topical dexamethasone for one month. The bandage contact lens is removed after epithelial healing.

To evaluate the effect of CXL on the corneal endothelium the following parameters obtained from the specular microscopy were evaluated: the endothelial cell density (ECD) and coefficient of variation (CV). The percentage of endothelial cell loss was calculated as follows: (preoperative cell count − postoperative cell count) /preoperative cell count* 100%.

Statistical analysis was done using the IBM SPSS version 22.0 (Armonk, NY: IBM Corp). The Shapiro Wilk test and Kolmogorov-Smirnov tests were used to verify the normality of distribution. Qualitative data were described using the number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. The Paired t-test was used to compare the means for normally distributed variables. Pearson correlation coefficient was used to examine the relationship between two variables. A P-value less than 0.05 was considered significant.

## Results

The study included 60 eyes of 60 patients (25 males and 35 females). The mean ± SD of age was 21.5 ± 3.5 years (range: 18-25). No complications were observed during the period of follow up.

**Specular microscopy data:**

The mean ± SD of preoperative ECD was 2604.25 ± 222.54 cell/mm$^{2.}$ Which decreased post operatively to 2584.53 ± 262.55 cell/mm2 but this was not statistically significant. (p=0.14).

The mean ± SD of preoperative CV was 39.72 ± 3.84%. This increased to 40.08 ± 4.17 % at three months but this change was not statistically significant. (p=0.16).

**CCT:** The mean ± SD of preoperative CCT was 492.88 ± 35.08 µm, while the mean postoperative CCT was 482.60 ±37.68 µm. The difference was statistically significant(p=0.001).

The mean± SD of intraoperative CCT was 386.42± 22.72 µm. The mean difference between the preoperative and intraoperative CCT was 106.47 µm ranging from 22 to 202 µm.

The percentage of endothelial cell loss ranged from -13 to 19%. The correlation between the difference in CCT and the percentage of endothelial cell loss was not statistically significant (r=0.19, p=0.16).

We divided the patients into two groups according to the mean intraoperative CCT. Group 1 had intraoperative CCT less than 386 µm and group 2 had intraoperative CCT ≥386 µm. There were no statistically significant differences in the preoperative CCT (p=0.9), pre and post operative ECD (p= 0.2&0.1), pre and post operative CV (p= 0.9&0.9), and percentage of endothelial cell loss and (p= 0.6) between the two groups. The pachymetric and endothelial cell data of both groups are presented in table (1)

**Table (1):** Demonstrates the mean, SD of ECD, CV, preoperative Pentacam and percentage cell loss in both groups.

| | Group 1 (n=32) | Group 2 (n=28) | P value |
|---|---|---|---|
| Intraoperative CCT (µm) | 368.16 ± 9.75 | 407.29 ± 13.11 | |
| Preoperative CCT (µm) | 490.94 ± 34.58 | 495.10 ± 36.14 | 0.9 |
| Peroperative ECD (cell/mm$^2$) | 2661.72 ± 189.43 | 2538 ± 242.14 | 0.2 |
| Postoperative ECD (cell/mm$^2$) | 2634.44 ± 220.79 | 2527.50 ± 297.22 | 0.1 |
| Endothelial cell loss (%) | -7 up to 8 | -13 up to 19 | 0.6 |
| Preoperative CV (%) | 39.06 ± 3.75 | 40.46 ± 3.87 | 0.9 |
| Postoperative CV (%) | 39.81 ± 4.35 | 40.39 ± 4.01 | 0.9 |

## Discussion

In the present study, the average EC loss was 1% and there were no statistically significant differences between the preoperative and postoperative ECD and CV. The effect of CXL on the corneal endothelium was the focus of many studies. While several studies showed the safety of the standard Dresden protocol on corneal endothelium[11-16], the studies of the effect of accelerated cross linking on corneal endothelium had contradictory results. Badawi, AE[17] found a significant reduction in ECD and a significant increase in CV at 3 and 6 months. The protocol followed in this study was the application of riboflavin 0.1% every 2 minutes for 10 minutes, then UVA irradiation of 10 mW /cm$^2$ for 9 minutes. Kymionis, GD et al.[18] did not find a significant change in the ECD and CV after one year of follow-up. Cingü AK et al.[19] found significant changes in the ECD, CV, and percentage of hexagonal cells in the first month postoperatively that became insignificant at six months for the ECD and three months for the CV. UVA irradiation is known to have a cytotoxic effect on human cells due to the formation of free radicals inside the cells.[20] In the standard Dresden protocol, the amount of UVA irradiation that reaches the corneal endothelium is about 0.18 mW/cm2, which is half the toxic level (0.35 mW/cm2)[19]. Accelerated cross linking uses a high-fluence protocol to reduce the exposure time according to Bunsen-Roscoe's law of reciprocity.[21] There are several modifications in the protocols of accelerated cross linking regarding the duration of riboflavin application, and UVA irradiation duration, and intensity. The difference in the treatment protocols together with different follow up periods can explain the inconsistency in the results of the studies. More studies are needed to determine the optimum protocol of accelerated cross linking that enhances the safety and efficacy of the procedure.

We measured the CCT intraoperatively after applying the riboflavin and found a significant reduction that ranged from 22 to 202 µm. The reduction in corneal thickness during crosslinking is documented in other studies, and it is attributed to corneal dehydration that occurs intraoperatively.[22, 23] Corneal thickness less than 400 µm is considered the most important risk factor for endothelial cell damage after CXL. However, some patients developed severe endothelial cell damage and corneal edema despite a preoperative thickness above 400 µm.[6, 24] In the present study, we did not find a significant difference in the endothelial cell loss between those with intraoperative CCT above or below 386 µm. Factors other than the corneal thickness may play a role in the extent of damage of endothelial cells, including excessive delivery of energy, inaccurate pachymetry and, the riboflavin used. Accelerated crosslinking results in a more superficial demarcation line than standard crosslinking[25, 26] that may have a protective effect on the corneal endothelium in cases with intraoperative CCT less than 400 µm, particularly when a shorter duration of treatment is used. More studies are needed to examine the corneal endothelial changes after accelerated crosslinking in thin corneas.

We found a statistically significant reduction in the CCT postoperatively. The reduction in CCT after cross linking was documented in other studies that used optical pachymetry.[27-29] Meanwhile, Wittig-Silva et al. did not find a significant difference in the CCT measured using ultrasonic pachymetry.[28] Several factors contribute to the reduction of CCT after CXL as epithelial thinning and keratocytes loss in the anterior stroma. The changes in stromal reflectivity after CXL can also affect the measurements by optical instruments like Pentacam or Orbsacn, resulting in a pseudo reduction in corneal thickness. Nonoptical pachymetry like confocal microscopy, anterior segment OCT or ultrasonic pachymetry are not affected by the change in stromal reflectivity and may be more accurate in measuring corneal thickness after CXL. Progressive corneal thinning is an important parameter in the detection of the progression of keratoconus. Establishing a baseline thickness postoperatively is important in the follow-up of these patients to detect possible progression after CXL.

In summary, our results showed that Accelerated crosslinking is safe in terms of corneal endothelial cell changes. The intraoperative reduction in CCT did not seem to affect the endothelial cells even in those with thickness less than 386 µm.

## Conclusion

Accelerated crosslinking is safe in terms of corneal endothelial cell changes. The intraoperative reduction in CCT seems to not affect the endothelial cells significantly even in those with thickness less than 386 µm.

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