

Comparison of central corneal thickness using ultrasound pachymetry during corneal collagen cross-linking

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Abstract

Purpose: To study central corneal thickness (CCT) variations during corneal collagen cross-linking (CXL) using ultrasound pachymetry.

Methods: Twenty patients (26 eyes) with progressing keratoconus undergoing riboflavin-UVA-induced CXL were involved in this study. Intraoperative CCT measurement using ultrasonic pachymetry was performed during the procedure. Measurements were obtained before operation, after epithelial removal, after riboflavin drop instillation, and after UVA irradiation.

Results: Mean CCT was 495 ± 56 and (450 ± 52) μm before and after epithelial removal, respectively. Mean CCT was (443 ± 42) and (411 ± 39) μm after riboflavin drop instillation and after UVA irradiation, respectively. Statistically significant decreases in CCT occurred between preoperation and after epithelial removal, after riboflavin drop instillation and after UVA irradiation. Twenty-six eyes from 20 patients undergoing CXL were divided into 2 groups (I with $\text{CCT} \geq 400$ μm after UVA irradiation and II with $\text{CCT} < 400$ μm after UVA irradiation). No statistically significant difference was noted between I and II for preoperative endothelial cell count, but statistically significant difference between I and II were noted for postoperative endothelial cell count. A statistically significant difference was evident between preoperative and postoperative endothelial cell counts in Group II ($P < 0.05$).

Conclusion: Performing CXL with the use of riboflavin and UVA irradiation resulted in a statistically significant decrease in CCT, even to a level where the corneal endothelium may be damaged.

Keywords: corneal collagen cross-linking; central corneal thickness; ultrasonic pachymetry; corneal endothelial cell

UV-light/riboflavin corneal collagen cross-linking (CXL) has recently become a new choice in ker-

atoplasty. CXL inhibits the progression of keratoconus, keratectasia post LASIK, and refractory corneal ulcer, reduces the possibility of corneal transplantation, and serves as a potential solution for the deficiency in donor corneas¹.

CXL is a relatively safe surgery. Many clinical trials have suggested that UV-light irradiation intensity ($3 \text{ mW}/\text{cm}^2$) at a standard dose causes no corneal transparency changes or corneal endothelial cell injuries for corneal thicknesses > 400 μm ⁴. However, other studies have reported a few cases of corneal endothelial cell injuries after CXL⁵⁻⁷, even though the corneal thickness was > 400 μm . The underlying reason is still unclear, but the incidence of such injuries is speculated to correlate with changes in corneal thickness during CXL. At present, a variety of methods are available for measuring corneal thickness; one of these uses the equation of the ultrasonic corneal pachymeter = the time of ultrasound passing through cornea \times ultrasonic speed in cornea. The precision of this method can be as high as 0.001 mm. The ultrasonic corneal pachymeter is now widely recognized as the gold standard of corneal thickness measurement and its accuracy and reproducibility have also been confirmed by many scholars. In this trial, we used A-mode ultrasound to monitor the changes in corneal thickness during CXL and to compare preoperative and postoperative corneal endothelial cell alterations.

Materials and methods

Study subjects

Twenty patients with progressive keratoconus (26 eyes) undergoing CXL between July and October 2012 in PLA General Hospital were randomly enrolled; they were 2 females and 18 males, aged

19.35±3.69 years on average (range from 14 to 27 years). The diagnostic criteria of keratoconus proposed by Rabinowitz⁹ were adopted: having a history of myopia and astigmatism; declining visual acuity; and corrected visual acuity < 20/20. At least one of the following positive signs was noted under slit-lamp examination: corneal stroma thinning, corneal ectasia, Fleischer ring, Vogt linear lesions, and epithelial or subepithelial scars.

Corneal topography revealed that diopter of was > 47 D; the diopter difference between 3 mm upper and below central cornea was >3 D; the diopter difference of central corneal anterior surface between two eyes was > 1 D. Inclusion criteria: corneal topography showed progressive aggravation of keratoconus; maximal corneal curvature was increased by 1 D or above within 6 to 12 months; or dominant dioptric spherical equivalent was elevated by 0.5 D or above. Exclusion criteria: corneal thickness < 400 μm; with a history of eye surgery, trauma, other corneal diseases, familial glaucoma, diabetes, connective tissue or mental illnesses, etc. The clinical study was approved by the Ethical Research Committee in our hospital. Informed consent was obtained from patients or their family relatives.

Preoperative and postoperative examinations

Preoperative and postoperative examinations included naked visual acuity, best-corrected visual acuity, intraocular pressure, corneal topography, corneal thickness, specular microscopy, Schirmer I test, slit-lamp examination and fundus examination, etc. All patients were administered 0.5% levofloxacin eye-drops three times a day for 2 to 3 days preoperatively.

Central corneal thickness (CCT) measurement: A-scan ultrasound pachymetry (UP-1000, Japan) was utilized to measure CCT preoperatively, after epithelial removal, after administration of riboflavin, and after ultraviolet radiation. Methods: the patients lay on a bed, were given 1 to 2 drops of tetracaine hydrochloride solution (50 g/L), and were told to look normally at the ceiling with both eyes. The ultrasound probe was positioned vertically to the cornea and touched the central cornea gently three times. The minimal measurement was used.

Corneal endothelial cell density measurement: specular microscopy (Topcon SP-2000, Japan) was

used to measure the density of corneal endothelial cells preoperatively and 1 month postoperatively. Methods: the patient sat in front of the equipment, rested the mandible on the bracket, and gazed at the indicator light. The images of central corneal endothelial cells were automatically acquired and processed to calculate the density of corneal endothelial cells.

Surgical approach

An area of epithelial cells (diameter=9 mm) on the corneal surface was mechanically removed. A piece of absorbent cotton soaked with 0.1% riboflavin solution (dissolved in 20% dextran) was placed over corneal surface, supplemented with riboflavin solution every 5 min to keep the cornea wet for 30 min. Later, slit lamp examination was performed to confirm the entry of riboflavin into the anterior chamber. The corneal tissues were irradiated (3 mW/cm²) by UV light at a wavelength of (370±5) nm and a beam diameter of 9 mm for 30 min, equivalent to 3.4 J. During irradiation, corneal surface was rinsed with 0.1% riboflavin solution and surface anesthetics every 5 min. After irradiation, antibiotic eyedrops were given and a bandage corneal contact lens containing 0.3% levofloxacin was worn until the corneal epithelia were healed.

Statistical analysis

SPSS 19.0 software was used for statistical analysis. Preoperative corneal thickness was compared with those after epithelial removal, droplet of riboflavin and irradiation by rank sum test. The operated eyes were divided into two groups (corneal thickness > 400 μm or <400 μm). In each group, endothelial cell densities were compared before surgery and 1 month postoperatively by a *t*-test. *P*< 0.05 was considered as the level of significance.

Results

Corneal thickness changes

Mean central corneal thicknesses were 495±56 μm preoperatively, 450±52 μm after epithelial removal, 443±42 μm after local administration of riboflavin, and 411±39 μm after ultraviolet radiation. The average difference in central corneal thickness before and after operation was 83±31 μm (ranging from 39 to 178 μm). The corneal thickness after epithelial removal, riboflavin administration, and ultraviolet ra-

diation significantly differed from the preoperative measurement (Table 1).

Corneal endothelial cell density changes

In group I ($n=17$), preoperative mean corneal endothelial cell density was $2630\pm 404/\text{mm}^2$ and $2427\pm 754/\text{mm}^2$ at 1 month postoperatively ($P>0.05$). In

group II ($n=9$), preoperative mean corneal endothelial cell density was $2803\pm 456/\text{mm}^2$, and $2277\pm 679/\text{mm}^2$ ($P<0.05$). No statistical significance was noted regarding endothelial cell density between two groups before surgery, but was observed at 1 month after surgery ($P<0.05$), as shown in Table 2.

Table 1 Changes in central corneal thickness during CXL (μm)

	Before operation	After epithelial removal	After riboflavin drop instillation	After UVA irradiation
Mean \pm SD	495 \pm 56	450 \pm 52	443 \pm 42	411 \pm 39
Maximum	623	579	543	529
Minimum	429	401	393	352
<i>u</i>		4.426	4.445	4.445
<i>P</i>		$P<0.01$	$P<0.01$	$P<0.01$

Table 2 Changes in corneal endothelial cell density in different groups post irradiation

Groups	Eyes	Preoperation	1-month post-operation	<i>t</i>	<i>P</i>
CCT \geq 400 μm	17	2630 \pm 404	2427 \pm 754	1.24	$P>0.05$
CCT<400 μm	9	2803 \pm 456	2277 \pm 679	2.22	$P<0.05$
<i>t</i>		1.46	2.10		
<i>P</i>		$P>0.05$	$P<0.05$		

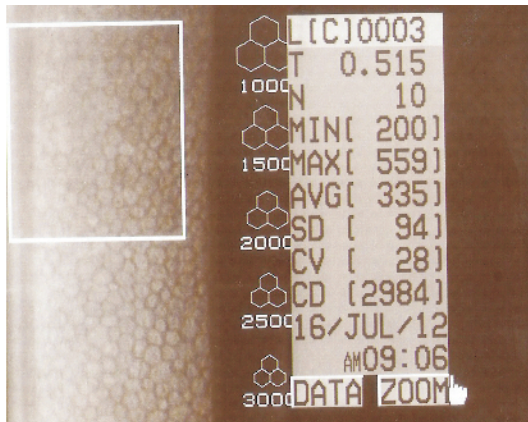


Figure 1 Endothelial cell analysis using corneal specular microscope before CXL

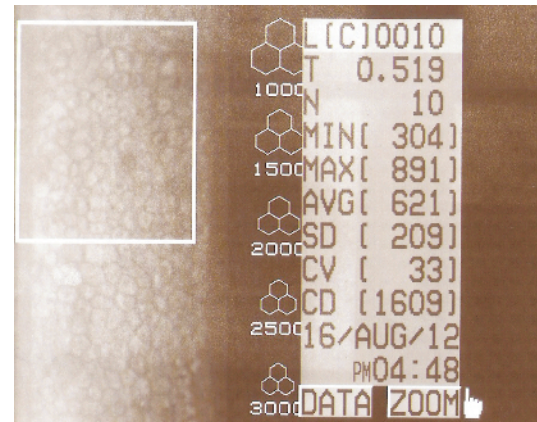


Figure 2 Endothelial cell analysis of the same patient using corneal specular microscope at 1 month postoperatively

Discussion

Gokhale et al⁵ reported one male case with keratoconus, aged 37 years, with a corneal thickness of 448 μm in the left eye, who underwent CXL. However, the patient presented with a severe decrease in visual acuity and corneal opacity at one day postoperatively. At six months postoperatively, a ring-shaped corneal scar was found in the operated eye. Corneal endothelial cell count was $1776/\text{mm}^2$ and $2978/\text{mm}^2$ for fellow eye. Bagga⁶ reported a 18-year

old patient with keratoconus who underwent CXL in the right eye. At three months after surgery, the corneal endothelial cell count was significantly decreased compared with the preoperative count. Sharma et al⁷ conducted a one year follow-up of 350 patients with keratoconus who underwent CXL and found that ten cases had permanent corneal edema whose preoperative corneal thicknesses were $> 400 \mu\text{m}$. The authors indicated that the irreversible corneal edema occurring in endothelial cell injury cases might be correlated with corneal thickness at-

tenuation. Therefore, corneal thickness <400 μm before surgery is a vital rather than a unique factor in the incidence of corneal endothelial injury complications after CXL. However, no studies have reported the intraoperative changes in corneal thickness.

In the present study, A-mode ultrasound was utilized to measure and observe CCT changes at various time points during the entire surgical procedure including before surgery, after epithelial removal, after instillation of riboflavin, and post irradiation. All patients presented with sharp decrease in CCT intraoperatively, and these values even declined to less than 400 μm in certain cases. For the four stages examined during CXL, CCT decreased most after epithelial removal and irradiation. Prior to operation, corneal endothelial cell density did not differ between two groups, while it significantly differed at 1 month post surgery. Corneal endothelial cell density in group I did not differ before and after CXL, whereas a significant decline occurred in group II.

Figures 1 and 2 show that the patients had a significant drop in endothelial cell density, and an increase in irregularly shaped and enlarged endothelial cells, indicating that corneal endothelial cell injury is possibly related to attenuation of the cornea during CXL, which results in an irradiation intensity that reaches the threshold of injury. Wollensak et al found that corneal endothelial cell injury is induced at an irradiation intensity of 0.36 mW/cm^2 during CXL. When corneal thickness was less than 400 μm , conventional irradiation intensity of 3 mW/cm^2 (corneal surface) during CXL may allow for irradiation energy (corneal endothelia) equivalent to the damage threshold^{10,11}. Although the patients did not present with clinical symptoms such as corneal edema, the long-term efficacy remains to be elucidated.

In traditional CXL, approximately 40 μm of corneal epithelium was removed, causing corneal attenuation. Kymionis et al¹² reported that 15 out of 19 patients with keratoconus had decreases in their corneal thickness of an average of 42.8 μm after removing the epithelia. In addition, persistent eye opening is likely to expose the cornea to the air and cause corneal attenuation by corneal dehydration. However, intact corneal epithelia can prevent the stromal layer from dehydration. Iwata et al¹³ estab-

lished rabbit eye models to analyze the resistance of various corneal layers to stromal dehydration; they found that the corneal epithelial layer showed the largest resistance, at 6.5 times that of the corneal lipid layer and 85 times that of the corneal tear film. However, the stromal layer provided essentially no resistance to corneal dehydration.

Corneal epithelial removal during CXL not only destroyed the intact corneal epithelial layer, but it also weakened the resistance of various corneal layers to stromal dehydration, which finally led to corneal attenuation. Kymionis reported that the corneal thickness of 15 patients was 415.7 ± 20.6 μm after epithelial removal and before dropping riboflavin, and then significantly decreased by 75 μm to 340.7 ± 22.9 μm at 30 min after topical application of a riboflavin drop. No significant difference was noted in CCT before and after ultraviolet radiation. Holopainen et al¹⁴ reported an opposite effect, where ultraviolet radiation cause a sharper decrease in CCT than did riboflavin treatment, probably because patients had to keep their eyes open during the whole ultraviolet radiation, which would lead to corneal stromal dehydration. In contrast, the patients were allowed to close their eyes during administration of the riboflavin solution, which is consistent with our current study. In our cases, the corneal surface was covered by absorbent cotton containing 0.1% riboflavin to retain moisture; this reduced the risk of corneal attenuation induced by corneal dehydration. Moreover, 0.2% riboflavin-dextran solution had to be dropped onto the corneal surface before and during irradiation process to allow for an adequate concentration of riboflavin within corneal stroma. Riboflavin-dextran solution has a relatively higher osmotic pressure compared to the extracellular fluid of keratocytes, which possibly leads to corneal dehydration. Wollensak et al¹⁵ supplemented three types of riboflavin in human and porcine cornea in vitro for 30 min and observed the changes in corneal thickness before and after the addition. The results showed that 0.2% riboflavin-dextran solution decreased human corneal thickness by 9.08% and porcine thickness by 7.32%.

In summary, corneal thickness was significantly decreased during CXL and even caused corneal en-

dothelial cell injury. Measurement of corneal thickness only before traditional CXL could not prevent the incidence of endothelial cell injury induced by corneal thickness attenuation. Since corneal attenuation mainly occurs after epithelial removal and ultraviolet radiation during CXL, we recommend that CCT should be accurately measured multiple times to observe its changing pattern. For the patients with CCT roughly equal to 400 μm preoperatively, removal of the epithelia should be avoided during CXL. In addition, the corneal surface should be kept moist to reduce corneal stroma dehydration and prevent corneal attenuation during administration of riboflavin drops. For patients with CCT < 400 μm intraoperatively, low osmotic riboflavin solution should be administered to induce corneal swelling¹⁶ and achieve a corneal thickness suitable for CXL.

Declaration: the authors are not involved in any conflicts of interests with pharmaceutical, equipment manufacturers stated in this study.

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