Original Article

Comparison of the findings of endothelial specular microscopy before and after corneal cross-linking

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Abstract

Background: To report the long-term findings of corneal cross-linking (CXL) with riboflavin drops on the corneal endothelial cell.

Materials and Methods: In this prospective non-randomized study, we aim to assess the long-term safety of CXL on the corneal endothelium for the treatment of progressive keratoconus, by endothelial specular microscopy. A total of 68 eyes of 42 keratoconus patients were selected. We checked the corneal thickness (with ultrasonic pachymetry), endothelial cell density, pleomorphism, and polymegathism (with specular microscopy) of the endothelial cells, before CXL and one year after this procedure.

Results: The mean ± SD of the preoperative and postoperative corneal thicknesses were 470 ± 40 μm and 469.8 ± 42 μm, respectively (p-value = 0.591). The mean ± SD of the preoperative and postoperative endothelial cell densities were 2753 ± 230 cells/mm$^{[2]}$ and 2699 ± 210 cells/mm,$^{[2]}$ respectively (p-value = 0.004). We found reduction in the endothelial cell density after CXL, but this reduction was less significant in a corneal thickness of less than 400 μm (which was treated with hypo-osmolar riboflavin 0.1% drops) compared to the corneal thickness of more than 450 μm. We did not find any significant differences in the cell shapes (pleomorphism) (p-value = 0.517), but the cell sizes (polymegathism) were changed after the procedure (p-value = 0.021).

Conclusion: We found a significant decrease in endothelial corneal cell density after CXL, but this reduction was low; also the size of these cells increased after CXL. We believe that other parameters besides the corneal thickness may be the determinant factors for the changing of cell density and cell size in corneal endothelial cells.

Key Words: Corneal cross-linking, endothelial cell density, keratoconus, specular microscopy, pleomorphism

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INTRODUCTION

Keratoconus is a progressive and degenerative disorder of the cornea.[1] Its incidence in the general population is reported to be approximately one in 2000.[2] In recent times, evidence has shown that collagen cross-linking (CXL) with riboflavin drops increases the biomechanical strength and stability of the cornea.[3]

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In this procedure on the cornea, additional cross-links can be induced within or between the collagen fibers using ultraviolet A (UVA) light and the photo-mediator riboflavin.[4] If this procedure is performed according to the standard protocols, it would be safe.[5] Despite the high safety profile reported, there are a few reports of adverse events after CXL, like persistent corneal edema.[3]

In some researches, in which hypo-osmolar riboflavin 0.1% was used for CXL in cornea with a thickness of less than 400 μm, it was found to be safe and effective.[4-8]

To assess the corneal endothelial cells, corneal specular microscopy is a noninvasive, photographic technique that allows visualization and analyzing of the corneal endothelium, with regard to the size, shape, and population of the endothelial cells.

The main purpose of this study is to assess the long-term safety of CXL on the corneal endothelium by endothelial specular microscopy comparing some parameters before and after the procedure.

MATERIALS AND METHODS

This prospective, non-randomized study was performed in the Feiz Eye Hospital (Isfahan, Iran). The Institutional Ethics Committee approved the study, and all patients signed a written informed consent after receiving a detailed description about the treatment. A total of 68 eyes of 42 progressive keratoconus patients, after 6 months of follow up, were enrolled, from January 2012 to February 2012. Some contraindications included central corneal opacities and severe dry eye, which could hinder re-epithelialization. Individuals who were pregnant, women in the breastfeeding period or those having systemic collagen vascular diseases should avoid undergoing CXL.[6] We selected patients with decreased corneal thickness (based on the comparison of corneal thickness during the last six months) and increased the Q-value on the topographic maps as changing criteria.[4] (during the last six months) before surgery, all patients had to discontinue contact lens. All the patients underwent preoperative and postoperative evaluations, including uncorrected (UCVA) and best corrected visual acuity (BCVA), slit lamp biomicroscopy, dilated fundoscopy, Goldman tonometry, corneal topography, specular microscopy with a non-contact specular microscope (Tomey EM-3000, Tomey Co., Japan) and ultrasound pachymetry (Tomey sp4, Tomey Co., Japan). The patients were examined postoperatively after one day, seven days, and one, three, and twelve months after the procedure. All the patients underwent the same CXL procedure UV-XTM, AcURA Co, Switzerland. Following the use of local anesthetic eye drops (Tetracaine hydrochloride 1%), a lid speculum was applied, and the eye was washed with saline. Next, the corneal epithelium was scraped with a hockey blade across an 8 millimeter diameter area. Subsequently, riboflavin 0.1% drop (Iso-osmotic: This solution is generated by diluting vitamin B2-riboflavin-5-phosphate 0.5% with dextran T500 20%, 402.7 mOsmol/L) for patients with corneal thickness of more than 400 μm, and hypo-osmolar (this solution is generated by diluting vitamin B2-riboflavin-5-phosphate 0.5% with physiological salt solution (sodium chloride 0.9% solution 310 m Osmol/L)) for patients with corneal thickness of less than 400 μm was applied to the cornea every 1 – 5 minutes, for 30 minutes. After 30 minutes, the slit lamp examination was done to ensure the riboflavin flare in the anterior chamber. Next, the eye was exposed to ultraviolet light with a wavelength of 365 – 370 nm. The light was irradiated from a distance of 5 cm for 30 minutes (3 mW/cm2, corresponding to a surface dose of 5.4 J/cm2). After completion of the procedure, antibiotic drops (ciprofloxacin 0.3%) were used and a bandage contact lens was placed. The postoperative medications included ciprofloxacin 0.3% drops four times a day, for seven days, and betamethasone eye drops six times a day, for one month. The bandage contact lens was removed one week after the procedure. We performed specular microscopy before CXL and one year after the CXL. The normal cell densities were between 1500 and 3500 cells/mm,[2] while more than 60% of the endothelial cells were six-sided.[9] The rate of polymegathism was represented by the coefficient of variation (CV). The CV values between 0.22 and 0.31 were considered normal.[10]

All the specular microscopy evaluations were conducted by the same operator. Statistical analysis of the specular microscopy parameters and other data (like age and corneal thickness) was performed with a paired *t*-test using the SPSS software version 18 (SPSS Inc., Chicago, IL).

RESULTS

The mean ± SD of the patients' age in this study was 20.7 ± 3.9 years (range: 13 – 31 years); 64.7% were male, and 35.3% were female. No persistent, early or late side effects were observed after the cross-linking procedure; also, no persistent corneal edema or delayed re-epithelialization was detected during the follow-up period. The mean ± SD of preoperative and

postoperative pachymetric values were 470 ± 40 μm and 469.8 ± 42 μm, respectively (p-value = 0.591). The mean preoperative and postoperative endothelial cell densities were 2753 ± 230 cells/mm^2 and 2699 ± 210 cells/mm^2, respectively. We found a significant reduction in the endothelial cell numbers after CXL (p-value = 0.004) [Table 1]. The least decrease in cell density was found in patients with corneal thickness less than 450 μm, while these patients had the thinnest cornea [Table 2]. The mean ± SD (interquartile range) of the coefficient variations of the endothelial cell size before and after the CXL were 32.72 ± 10.14 (25–82) and 40.21 ± 9.70 (8–83), respectively (p-value = 0.021) [Table 3]. The mean ± SD of the preoperative percent of hexagonal cells (pleomorphism) in the endothelium was 54.14 ± 6, and the postoperative percent was 54.55 ± 5 (p-value = 0.517) [Table 4].

Dividing the patients into two groups based on their age (≤18, and > 18 years) and comparing the endothelial cell density, we found a significant decrease only in the older age group (p-value = 0.01) However, in the younger-than-18 age group, we did not find a significant decrease in cell density after one year (P value = 0.10). However, there was no statistically significant difference between the two groups in corneal thickness (*p*-value = 0.19 in younger-than-18 year and 0.49 in the older-than-18 year age group) [Table 5].

DISCUSSION

The purpose of this study was to examine the corneal endothelial cells before and after CXL. Essentially, we found that the corneal endothelial cell density was significantly decreased after corneal cross-linking, but the mean of this reduction was negligible, about 60 cells (p-value = 0.004). In one study done by Vinciguerra *et al.*, on 28 eyes, the mean baseline endothelial cell count decreased after 24 months, but this reduction was not statistically significant.[11]

Another similar study that was done in 2009, on 30 eyes, also showed the same conclusion in endothelial cell density, but, after one month, the corneal thickness was lower than the preoperative thickness, however, after six months, the corneas had regained their original thickness. We too found that the corneal thickness did not change after one year (p-value = 0.591).[12] Also, Goldich, in another study, did not find a significant change in corneal thickness.[7]

On the other hand, Sharma and colleagues reported a case series of patients undergoing CXL. Postoperative corneal edema was identified in 10 patients, in whom specular microscopy was not successful, and finally two patients underwent penetrating keratoplasty.

Table 1: Preoperative and postoperative studied parameters

Parameter (*n*=68)	Preoperative	Postoperative	*P* value
Age	20.76±3.98 (12-31)		
Male/female	44/24		
Corneal thickness	470.88±46.79 (364-591)	469.94±49.55 (352-590)	0.591
CV	32.72±10.14 (25-82)	40.21±9.70 (28-83)	0.021
CD	2754±230.49 (1986-3211)	2699.6±210.97 (1980-3194)	0.004
Pleomorphism	54.15±6.33 (40-70)	54.56±5.09 (40-7)	0.517

Data presented as median (IQR). *P* values calculated by version 16.0, SPSS Inc., Chicago, IL. CV: Coefficient of variation

Table 2: Comparison of endothelial cell density based on corneal thickness in 68 eye samples of the study population

Corneal thickness (μm)	*N*	Age (years)	Cell density (cells/mm^2)		*P* value
			Preoperative	One year after operation	
370-400	7	20.57±4.43 (17-30)	2643.9±256.75 (2133-2942)	2676.7±89.38 (2556-2774)	0.714
400-450	14	19.43±2.74 (14-22)	2667.1±163.44 (2404-2937)	2614±150.52 (2390-3000)	0.285
450-500	32	21.37±4.19 (12-31)	2750.2±240.76 (1986-3116)	2692.9±239.57 (1980-3080)	0.017
>500	15	20.80±4.33 (12-31)	2894.5±195.06 (2564-3211)	2804.1±205.32 (2446-3194)	0.012

Data presented as mean±SD (interquartile range). Median (IQR). SD: Standard deviation

Table 3: Comparison of mean coefficient of variation of endothelial cell size based on corneal thickness in 68 eye samples of the study population

Corneal thickness (μm)	*N*	CV of endothelial cell size		*P* value
		Preoperative	One year after operation	
370-400	7	39.29±9.72 (30-58)	38.43±8.9 (28-54)	0.713
400-450	14	36±4.45 (25-51)	41.29±9.1 (31-57)	0.032
450-500	32	37.34±10.22 (25-82)	39.53±10.96 (29-83)	0.043
>500	15	39.4±12.7 (28-79)	41.47±8.28 (31-58)	0.570

Data presented as mean±SD (interquartile range). Median (IQR). CV: Coefficient of variation; SD: Standard deviation

Table 4: Comparison of mean±SD of the percent of hexagonal cells (pleomorphism) in the endothelium based on corneal thickness in 68 eye samples of the study population

Corneal thickness (μm)	*N*	Percent of hexagonal cells (pleomorphism) (%)		*P* value
		Preoperative	One year after operation	
370-400	7	55.71±8.38 (45-70)	55.71±9.32 (40-70)	1.000
400-450	14	56.43±7.45 (40-70)	53.43±5.94 (40-60)	0.205
450-500	32	53.19±5.01 (40-60)	54.84±3.23 (50-60)	0.037
>500	15	53.33±6.72 (40-65)	54±5.41 (5-65)	0.499

Data presented as mean±SD (interquartile range). Median (IQR). SD: Standard Deviation

Table 5: Comparison of endothelial cell density based on age in 68 eye samples of the study population

Age	Pre-operative cell density	Cell density after 1 year of operation	*P* value	Pre-operative corneal thickness	Post operative corneal thickness	*P* value
≤18 (*n*=15)	2783.9±213.67 (2434-3211)	2734.9±189.9 (2483-3194)	0.101	460.67±48.60 (364-538)	462.33±49.93 (370-539)	0.190
>18 (*n*=53)	2745.5±236.28 (1986-3182)	2689.6±217.21 (1980-3118)	0.014	473.77±46.33 (365-591)	472.09±49.70 (350-590)	0.449

Data presented as mean±SD (interquartile range). Median (IQR). CV: Coefficient of variation; SD: Standard deviation

This case series reports the possibility of corneal endothelial damage, with visually significant corneal edema, after CXL treatment.[3]

In a case report, corneal endothelial damage after collagen cross-linking treatment was reported. The cell density after resolution of edema in this patient was 1776 cells/mm^2 in the affected eye compared to 2978 cells/mm^2 eye in the untreated fellow eye. This study concluded that corneal thickness was not the only determining factor for corneal endothelial damage after the cross-linking procedure.[13] This finding was similar to our study, because we found more decrease in cell density in groups with a thicker cornea [Table 2]. We found more reduction in CD (cell density) in corneal thickness of more than 450 μm. (*p*-value in the group with 450 – 500 μm corneal thickness was 0.017 and in > 500 μm was 0.012).

In the present study, a change in the percent of hexagonal cells was not significant and possibly not affected by CXL. A change in CV (showing polymegathism) was significantly increased after CXL (preoperative CV = 32.7 ± 10 and postoperative CV = 40.2 ± 9.7, and *P* value = 0.021).

In a similar study on 14 patients done by Goldich *et al*., no morphological abnormalities or density changes in endothelial cells were reported after 12 months.[7]

In the other study done in Turkey evaluating the short-term specular microscopic findings after CXL, for progressive keratoconus, no significant changes in endothelial cell counts were reported, although they found some changes in the morphological parameters. The mean pleomorphism value decreased significantly from 49 ± 5.80 preoperatively to 39.88 ± 6.24 postoperatively (p-value = 0.01).[14] In the present study we did not find any significant change in pleomorphism (preoperative 54 ± 6.33 to postoperative 54.56 ± 5 and *P* value = 0.517).

In some researches in which hypo-osmolar riboflavin was used for CXL in a cornea with the thickness of less than 400 μm, it was found to be safe and effective.[4,8] We used hypo-osmolar riboflavin for corneal thickness of less than 400 μm and found no significant reduction in the cell density of this group of patients. On the other hand, CXL with iso-osmolar riboflavin in patients with a thicker cornea (more than 450 μm) significantly decreased the cell density. In fact we found that hypo-osmolar riboflavin was safe for endothelial cells with corneal thickness less than 400.

In patients younger than 18 years, reduction in endothelial cell density was statistically insignificant (p-value = 0.1) compared to the patients older than 18 years (p-value = 0.014). This finding would be acceptable, because in other researches, the corneal endothelial cells reduced with aging.[15]

In the present study, we did not find any changes in the corneal thickness and this was similar to other researches.[16-18]

CONCLUSION

We found that the endothelial corneal cell density decreased significant after CXL, but this reduction was low, and the size of these cells (CV) increased after CXL.We believe that other parameters besides the corneal thickness could be the determinant factors for the changing of cell density and cell size in corneal endothelial cells.

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