Effect Of Accelerated Corneal Collagen Cross Linking (CXL) On Corneal Endothelium

Abstract

Aim: To study the effect of accelerated collagen cross linking on corneal endothelial cell count and morphology in patients with progressive keratoconus.

Design: Prospective non randomised interventional study.

Material & Methods: 40 consecutive eyes that underwent corneal collagen cross linking with riboflavin (CXL) for keratoconus were included in the study. Corneal endothelial cell count and morphology were compared before CXL and after CXL.

Results: In this study we found that with accelerated collagen cross linking change in endothelial cell count and coefficient of variance was statistically significant with P value of P<0.001 and P<0.004 respectively when compared preoperative values.

Conclusion: There is effect of accelerated collagen cross linking on the corneal endothelial cell and morphology which needs to be further studied.

Keywords: Collagen; Cross linking

Research Article

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Introduction

Keratoconus, which was first described in detail in 1854, it derives from the Greek words Kerato (cornea) and Konos (cone) [1]. The main goal of treatment of keratoconus has changed over the last few years from that focused mainly on improvement of visual acuity to an array of newer modalities focused on the prevention of progression of the disease. In keratoconus ectasia occurs as a result of reduced biomechanical strength of the cornea possibly related to reduce interfibrillar cross-linking. The only modality which targets the pathogenic mechanism is collagen cross linking. Wollensak et al. [2] introduced corneal collagen cross linking using riboflavin (CXL) and ultraviolet A radiations for the treatment of progressive keratoconus .Endothelial cell density and morphology is a key indicator when evaluating and maintaining corneal health. The degree of endothelial cell loss can be documented with specular microscopy as an increase in individual cell surface area and a decrease in the endothelial cell density for the cornea [3]. However, the effect of accelerated collagen cross linking on the corneal endothelial cell and morphology have not been reported much previously in the literature. This study was undertaken to study effect of accelerated collagen cross-linking on corneal endothelial cell count and morphology in keratoconus patient.

Material and Methods

Forty consecutive eyes of forty patients with progressive keratoconus and preoperative corneal thickness of more than 400 micron were treated with accelerated collagen cross linking. Informed consent was obtained from all patients after the nature and possible consequences of the study and procedure were fully explained. Study was registered with institutional review board and an approval from the ethical committee was taken. The study included patients who had progressive keratoconus (Increase in the maximum keratometry reading >1D for 1year, spherical refractive error by 0.50D for 6 months & Astigmatism by 1.0D 6 months), age 14-45 years, deteriorating visual acuity, contact lens intolerance and cornea thicker than 400 micron documented by pachymetry.

All cases of progressive keratoconus with less than 400 microns, any history of herpetic keratitis or autoimmune disease were excluded from the study. Patients underwent a complete ophthalmic examination which included a detailed history taking, best corrected visual Acuity (BCVA) for distance was measured using Snellen's chart and logMAR chart, Anterior segment examination by Slit lamp biomicroscopy (Topcon SL 1E, Topcon Corp, Japan), a dilated fundus examination with +90D Slit lamp biomicroscopy (Topcon SL 1E, Topcon Corp, Japan) and by indirect ophthalmoscope (Heine Sigma 150 HC, Heine, Germany) with a +20D condensing lens, Pachymetry was done with (Tomey pachymetry SP 2000), Topography was done using (Keraton Scout Optikon 2000), Keratometry was done with Auto-Kerato-refractometer (Topcon KR – 8800), tonometry was done with Non-contact tonometer (Topcon CT – 80 Computerised Tonometer) and specular microscopy was done with (Tomey EM–3000,USA). Keratoconus was graded using the Rabinowitz/ Mc Donnel classification.

The treatment procedure was done under sterile conditions in the operating room. Proparacaine 0.5% Eyedrops (sunways pharma) were applied for preoperative local anesthesia. The central 8 mm of the corneal epithelium was cautiously removed using 20% alcohol with help of 8 mm well for 30 second. As a photo sensitizer, riboflavin 0.1% solution (Vibex Rapid-Riboflavin 0.1% with Hydroxypropyl Methylcellulose) was applied every 1 minute for 10 minutes before the irradiation. The cornea is then

exposed to near ultraviolet radiant energy from a solid-state UV lamp source [light-emitting diode (LED)] at a wavelength of 365-370 nm (in the UV-A spectral band) (avedro CXL) with continuous mode at an irradiance ("exposure dose rate") of 30 mW/cm^2 for 3 minutes to achieve a total radiant exposure ("total dose") of 5.4 J/cm^2 [4]. At the end of the procedure, a bandage contact lens was placed and the lid speculum was removed from the eye. Postoperatively, moxifloxacin hydrochloride 0.5% (Vigamox, Alcon, TX) eye drops two times a day for 10 days and preservative free artificial tears four times a day for one month were prescribed. Bandage contact lens was removed when epithelialization was complete, usually after 4 days. After complete epithelialization loteprednol etabonate eye drop 0.5% (Lotepred, Sun Pharma) were prescribed four times a day and were tapered for the next three weeks.

All patients were examined postoperatively at 1st, 3rd and 6,12m after the treatment and following examination was carried out which included uncorrected visual acuity (UCVA), and best corrected visual acuity (BCVA), slit-lamp biomicroscopic examination, corneal topographic analyses, keratometry, pachymetry and status of the corneal endothelium with specular microscope. Post-operatively, in all patient eyes, the endothelium was photographed and evaluated in vivo using a Topcon SP3000p non-contact autofocus specular microscope (Topcon Corp, Tokyo, Japan) at 3m, 6m and 24m. Images of the central corneal window were analyzed and manually corrected and three measurements of ECD were averaged.

Statistical Methods

A descriptive and inferential statistical analysis has been carried out in the present study. Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. Paired proportion test has been used to find the significance for BCVA/UCVA. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data .

Results

In our study out of 40 patients that were selected, only 1 patient (2.5%) had age < 10yr,22 patients (55%) belonged to age group between 10-20yr, 16 patients (40%) belonged to age group between 21-30yr and 1 patient (2.5%) had age >30yr. Male and female distribution was 1:1. Preoperative mean UCVA (Log MAR) was 0.86±0.27. And the postoperative mean UCVA at 12 months was 0.66±0.30 with a P value <0.001(Table 1a). The preoperative mean BCVA (Log MAR) was 0.14±0.17 and postoperative mean BCVA at 12 months is 0.062±0.11 with a P<0.001(Table 1a) Mean baseline flattest meridian keratometry was 44.59±2.53. One month after the procedure it was 44.08±2.57; at 3 month it was 43.68±2.51, at 12 month 43.39±2.45. The difference between baseline and 12 months was significant with P<0.001 (Table 1b). Mean baseline steepest meridian keratometry was 48.50±3.56. One month after the procedure it was 47.85±3.51, at 3 month it was 47.32±3.33, at 12 month was 47.00±3.28 Difference between baseline and 12 months was significant with P<0.001 (Table 2a). Mean baseline average keratometry was 46.54±2.79. One month after the procedure it was 45.96±2.77, at 3 month it was 45.50±2.65, at 12 month 45.20±2.59. Difference between baseline and 12 months was significant with P<0.001 (Table 2b). Mean baseline endothelial cell density was 2915.15±209.49 cell/mm^2.One month after the procedure, it was 2851.55±207.87 cell/mm^2, at 3 months 2848.03±207.78 cell/mm^2, at 12 months 2840.90±209.17 cell/mm^2. Difference between baseline and 12 months was significant with P<0.001(Table 3a). The mean baseline coefficient of variation was 30.85±3.48.One month after the procedure; it was 31.25±3.55, at 3 months 31.25±3.55, at 12 months 31.23±3.50. Difference between baseline and 12 months was significant with P<0.004 (Table 3b). Pachymetry with thinnest point did not change significantly at the end of 12m (Table 4).

Table 1a: An evaluation on UCVA and BCVA with Mean±SD and P value (LogMAR).

	Pre-Op	1 Month	3 Month	12 Months
UCVA				
Mean ±SD	0.86±0.27	0.82±0.29	0.70±0.31	0.66±0.30
P value from Pre-op	-	0.025*	<0.001**	<0.001**
BCVA				
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 1b: An evaluation of Sim k Flat.

Time Points	Min-Max	Mean±SD	Difference	P Value
Pre-Op	39.91-50.29	44.59±2.53	-	-
1 month	39.80-49.97	44.08±2.57	0.513	<0.001**
3 months	39.51-49.72	43.68±2.51	0.909	<0.001**
12 months	39.35-49.67	43.39±2.45	1.198	<0.001**
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 2a: An evaluation of Sim k Steep.

Time points	Min-Max	Mean ± SD	Difference	P Value
Pre-Op	42.91-56.67	48.50±3.56	-	-
1 month	42.62-55.71	47.85±3.51	0.645	<0.001**
3 months	42.08-54.17	47.32±3.33	1.180	<0.001**
12months	41.72-53.83	47.00±3.28	1.494	<0.001**
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 2b: An evaluation on Average values of K.

Time points	Min-Max	Mean ± SD	difference	P value
Pre-Op	41.41-53.18	46.54±2.79	-	-
1 month	41.21-52.22	45.96±2.77	0.579	<0.001**
3 months	40.80-51.07	45.50±2.65	1.045	<0.001**
12 months	40.54-50.71	45.20±2.59	1.346	<0.001**
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 3a: An evaluation based on ECD.

Time Points	Min-Max	Mean ± SD	Difference	P Value
Pre-Op	2,452.00-3446.00	2915.15±209.49	-	-
1 month	2,409.00-3392.00	2851.55±207.87	63.600	<0.001**
3 months	2,397.00-3382.00	2848.03±207.78	67.125	<0.001**
12 months	2,394.00-3379.00	2840.90±209.17	74.250	<0.001**
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 3b: An evaluation based on CV.

Time points	Min-Max	Mean ± SD	Difference	P Value
Pre-Op	25.00-40.00	30.85±3.48	-	-
1 month	25.00-40.00	31.25±3.55	-0.400	0.002**
3 months	25.00-40.00	31.25±3.55	-0.400	0.002**
12 months	25.00-40.00	31.23±3.50	-0.375	0.004**
Mean ±SD	0.14±0.17	0.14±0.18	0.086±0.15	0.062±0.11
P value from Pre-op	-	0.971	<0.001**	<0.001**

Table 4: An evaluation based on Pachymetry.

Parameters	Pre Op	Post op 1 Months	p Value	Post op 3 Months	Post op 12 m	P Value
TP	465.9483	455.6897	0.0001	455.6897	455.2586	0.738

Discussion

The CXL is a new treatment modality for keratoconus patients. Ultraviolet-A irradiation has a well-known cytotoxic and pro-apoptotic potential in human cells. It causes the formation of free radicals such as singlet oxygen, superoxide and hydrogen peroxide species in endothelial cells, which can consequently result in apoptosis. Experimental studies demonstrated that the combined use of UVA irradiation with riboflavin can decrease the toxic effects of the treatment by 10 times compared with the use of UVA alone. Corneal endothelial changes after the standard CXL with UVA and riboflavin in progressive keratoconus were previously evaluated in different investigations.

However, the effect of accelerated collagen cross linking on the corneal endothelial cell and morphology have not been reported much previously in the literature. This study was undertaken to study effect of accelerated collagen cross-linking on corneal endothelial cell count and morphology in patient with progressive keratoconus. The K_{max} value is a guide to evaluate success of the CXL procedure since it shows the severity of the KC. The K_{max} value was reported to be decreased after CXL [5-7] and in this study average K value and steep K value decreased with a significant P value. In this study UCVA showed improvement significantly at 12 months with mean of 0.66±0.30 and P value<0.001. However in Yasin Çınar et al. [8] study there was no statistically significant change in the mean UCVA at 6 months (p=0.332).

In our study BCVA showed improvement significantly with mean of 0.062±0.11 and P value <0.001. In this study endothelial cell density was decreased at 1 month, 3 month, 12 month with P<0.001 when compared with their preoperative values. In Cingü AK et al. [9] study ECD values were decreased at 1st week, at 1st month and at 3rd month of follow-up (p=0.006, p<0.001, and p=0.014, respectively) compared to preoperative values. At 6 months, there was no significant difference (p=0.36). In this study percentage of endothelial cell loss seen was 2.53%. In this study coefficient of variation was increased at 1 month, 3 month and at 12 month (p<0.002, p<0.002, p<0.004 respectively) compared to preoperative value. In Cingü AK et al. [9] study the mean percentages of CV significantly increased at 1st week and at 1st month postoperatively when compared with the preoperative measurements (P=0.006 and p=0.001, respectively) whereas turned toward preoperative values at 3rd month and 6 month (p=0.22, p=0.25 respectively.

Differences seen in this study compared to other studies in corneal endothelial cell count and coefficient of variance may be due to the more intense UVA irradiance (30 mW/cm^2) used by us according to guideline of machine and two patients in our study showed endothelial cell loss of 4.6% and 4.8% which is greater than average seen in this study which might have affected the results. In Cingü AK et al. [9]. Study surface irradiance of 18 mW/cm^2 was used and in Yasin Çınar et al. [8] surface irradiance of 9 mW/cm^2. In the confocal microscopic studies [6,10,11] with the conven-tional CXL, decreased keratocyte density was observed 1 month after the CXL. Three months after the procedure, keratocyte repopulation was observed in the treated area and continued progressively to complete over the 6 to 9 months with no endothelial cell damage. Confocal microscopic study in the accelerated CXL showed similar results with the conventional CXL method[25].Complications of the conventional CXL procedure are rarely seen, including sterile infiltrates, infectious keratitis, corneal scarring, endothelial damage, persistent epithelial defects and corneal edema [12-14]. We did not observe any serious complication mentioned above in our study. We noted that there was statistically significant transient decrement in the ECD at the 1st month,3m and 12m as previously reported [15,16]. The number of the patients, short follow up duration and lack of comparison between conventional CXL method and accelerated CXL method were limitations of our study.

Conclusion

Our preliminary results showed that accelerated CXL procedure for 10 min was effective to stop the KC progression and as well as improvement in visual acuity and keratometric values at a short time period. Longer follow up with larger patients and comparative study with conventional CXL procedure is recommended.

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