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Research Paper

Comparison of Corneal Densitometry Measured by Pentacam before and after Collagen Cross Linking (CXL) in Patients with Keratoconus (KCN)

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Abstract

Introduction: Keratoconus (KC) is the most common corneal ectatic disorder with a prevalence of about 1 in 2,000 people. KC is a progressive, non-inflammatory, almost bilateral and asymmetric of corneal. Corneal collagen cross-linking (CXL) with riboflavin and ultraviolet- A (UVA) is a new technique of mechanical stability of the cornea and prevention of keratoconus progression.

Purpose: Determining the relationship between changes in corneal density before and after collagen cross-linking surgery in patients with keratoconus

Materials and Methods This prospective study was performed on 45 eyes of 23 patients with keratoconus undergoing corneal collagen cross-linking treatment at Imam Khomeini Hospital in Ahvaz in 2019. Corneal densitometry (CD) and pachymetry were measured using Pentacam before CXL and at 3, 6 and 12 months after CXL. Also, all of the patients were evaluated in terms of best corrected visual acuity (BCVA), during follow-up.

Results: In this study, 23 people including 12 men (54.2%) and 11 women (45.8%) with a mean age of 25.2 ± 4.64 years in the age range of 18-34 years participated. The mean value of corneal density in total layer over the annular diameters 0-2 mm was 19.10±5.58, 22.31±5.64, 24.28±6.37 and 20.89±5.73 for before, 3 months, 6 months and 12 months after CXL, respectively (P = 0.001). The mean value of corneal density in ant layer over the annular diameters 0-2 mm was 22.38±8.38, 27.46±9.63, 28.07±11.28 and 24.53±9.21for before, 3 months, 6 months and 12 months after CXL, respectively (P = 0.001). The mean value of corneal pachymetry in thinnest location was 426.2±34.7 µm, 412.5±42.6 µm, 419.2±37.3 µm and 428.3±36.9 µm for before, 3 months, 6 months and 12 months after CXL, respectively (P = 0.392). The pre-CXL value of BCVA was significantly lower than the post-CXL values in 12 months (0.10 ± 0.19, 0.15 ± 0.10 and 0.019 ± 0.15, respectively, P<0.0001). In general, Densitometry values increases and corneal thinnest location decreses during the 3-6 months after CXL and then return to baseline preoperative values in 12 months. There was a significant improvement in best corrected visual acuity (BCVA) before and 12 months after surgery (P<0.0001).

Conclusions: CXL can increase corneal densitometry and decrease corneal thinnest location during the postoperative 6 months period. Densitometric values and corneal thickness return to baseline preoperative values to a large extent during 6-12 months postoperatively. The patient's BCVA increased over 12 months but no correlation was observed between this increase and densitometric changes.

Keywords: Keratoconus, Collagen cross-linking, Pentacam, Corneal Densitometry.

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1. Introduction

Keratoconus (KC) is the most common cornea ectatic disorder with a prevalence of about 1 in 2,000 people. KC is a progressive, non-inflammatory, almost bilateral and asymmetric of corneal ectasia [1]. Some studies have shown that the prevalence of this disease is higher in women, while some other studies have shown that the prevalence is the same in both gender. The disease usually appears in the second decade of life, naturally around puberty, and usually progresses to the fourth decade [2, 3]. The pathophysiology of KC disease includes structural changes in collagen, disruption of their arrangement, changes in intercellular matter and necrosis and apoptosis of keratocytes in the central part of the stroma and Bowman's layer, all of which indicate a weakening of corneal tissue [4].

Recently, a decrease in the bonds between collagen molecules has been observed in patients with KC compared to normal individuals. Most patients have refractive errors myopia (nearsightedness) and astigmatism that will improve over time and with the addition of other irregular astigmatism corrected with glasses does not respond [5]. Corneal transplantation to correct refractive errors and establish tectonic strength of the cornea has no role in the disease process. Collagen cross-linking is currently the only promising treatment that can slow the progression of the disease. Corneal collagen cross-linking using riboflavin and ultraviolet A is a new treatment for KC. Interestingly, riboflavin is also present in the lens, which absorbs ultraviolet rays and prevents retinal damage. In long-term, the lens also cross-links and turns yellow [6].

collagen cross-linking is used not only to prevent the progression of KC but also to inhibit the progression of corneal ectasia after refractive surgery, corneal wound healing and corneal edema [7]. Corneal Cross-Linking (CXL) was introduced in 1997 by Mr. Sporel and Seiler and its standard protocol was completed over time. The effect of collagen cross-linking therapy on the development of KC was first reported by Wolensak et al. By cross-linking, an additional covalent bond is created between the collagen molecules and the collagen scaffold is stabilized [8]. Collagen cross-linking-associated corneal haze may occur in 10-90% of cases and is due to keratocyte proliferation, pressure changes in sternal swelling, collagen-proteoglycan interventions, or hydration of glycosaminoglycans [9].

Scheimpflug imaging has a large number of applications in corneal diagnosis. Corneal and anterior segment tomography based on a rotating Scheimpflug camera allows for imaging of both the anterior and posterior corneal surfaces. It is possible to measure the amount of backscattered light in the different regions of the cornea. A normal cornea is expected to scatter light mostly in the interfaces air/cornea and cornea/water where there are higher differences in light refraction indices with only minimum scatter by the structures of corneal tissue .Densitometry is expressed in gray scale units, ranging from a minimum light scatter of 0 (maximum transparency) to a maximum light scatter of 100 (minimum transparency). [9].

Studies have shown that weakening of corneal tissue in the keratoconus causes changes in corneal parameters such as corneal strength and corneal astigmatism [10-12]. Some studies have also shown that CXL causes changes in corneal thickness, corneal densitometry, anterior chamber angle, anterior chamber volume and depth compared to preoperative [13, 14]. The aim of this study is to comparing the changes in corneal densitometry, thinnest location and BCVA in patients with keratoconus using Pentacom before and after collagen cross-linking.

The aim of this study was to evaluate corneal densitometry before and after collagen cross-linking in patients with KC.

2. Materials and Methods

The present study is a prospective study conducted on Keratoconus patients referred to the ophthalmology clinic of Imam Khomeini Hospital. According to a study by Shen et al., densitometric changes of the total layer after CXL are 18.47±1.81. The sample size was calculated based on the following formula:

$$n = \frac{2^2_{1-\alpha/2} \times \sigma^2}{d^2}$$

Exclusion criteria included pregnancy and lactation, patients with a history of corneal surgery or corneal hydrops, patients with any complications during and after surgery, people with diabetes or collagen tissue diseases, severe dry eye syndrome, visual acuity ≥ 20.25 and cornea thickness (TCT) less than 400 microns.

Before surgery, corneal densitometry and pachymetry was measured by Pentacam and was repeated at 3, 6, and 12 months after the surgery. A new Pentacam software module enables corneal densitometry to be analyzed. Corneal densitometry can be measured in 4 annular zones of the cornea. The zones are centered on the apex of the cornea, the first zone being 2 mm in diameter, the second zone 2 to 6 mm the third zone 6 to 10 mm, and the fourth zone 10 to 12 mm.. The densitometry measurement can be provided for the anterior (first 120 mm), central (from the first 120 mm to the posterior 60 mm), and posterior parts (60 mm) of

the cornea. The "total layer" refers to the volume between the epithelium and endothelium of a cornea. For each eye, the measurements on the central annuli of diameters from 0 to 2 mm (Φ0–2 mm) and from 2 to 6 mm (Φ2–6 mm) of the corneas' anterior layer, central layer, posterior layer, and the total layer were analyzed. All procedures were performed by 1 surgeon.

On the day of surgery, pilocarpine1% eye drop was used to miosis the pupil and reduce damage to the lens and retina. Under sterile conditions and after topical anesthesia with tetracaine0.5% eye drop the central corneal epithelium was removed with a diameter of 9 mm, then a drop of 0.1% riboflavin was applied on the surface of the cornea every 2 to 3 minutes for 30 minutes. After ensuring the entrance of riboflavin into the anterior segment with use of a slit lamp, ultraviolet radiation with a wavelength of 370 nm and a power of 3mw /cm^2 at a distance of 1 cm from the cornea was started for 30 minutes at a dose of 4.5 joules per square centimeter. Riboflavin drops were applied on every 2-3 minutes. A bandage contact lens is then placed on the cornea until the epithelium is completely healed. Prophylactic antibiotics and topical betamethasone0.1% eye drop were used for four weeks after epithelial repair.

2.1 Statistical analysis

Data analysis performed by SPSS version 22 (SPSS Inc., Chicago, IL, USA), and p-value less than 0.05 was statistically significant. First, the normal distribution of the data was assessed by the Kolmogorov–Smirnov test and homogeneity of variances by Levene's test. Depending on the data distribution, unpaired t tests and Mann–Whitney U tests were used.

In quantitative variables, the mean was used to describe the data center and the standard deviation was used to describe the scatter of the data. Frequency and percentage were used to describe qualitative variables. Due to the non-compliance of the data with the normal distribution, in this study, more non-parametric tests were applied to analyze the results. Wilcoxon test and the Spearman correlation test were also used to analyze the data. Linear mixed effect test or repeated measures analysis with random effect was used to compare density changes at different times.

2.2 Ethics

The study was accepted by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. (IR.AJUMS.REC.1398.062). Written, informed consent was achieved from each patient.

3. Results

In this study, 23 patients including 12 (54.2%) men and 11 women (45.8%) with a mean age of 25.2 ±4.64 years participated in the age range of 18-34 years. The results of measuring corneal densitometry before and after cross-linking are presented in Table 1.

Table 1. Changes in Densitometry Values of Each Position of a Cornea before & after CXL

Variables	Preoperation (1)	3 month Postoperation (2)	6 months Postoperation (3)	12 months Postoperation (4)	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Anterior layer (Φ 0–2 mm)	22.38±8.38	27.46±9.63	28.07±11.28	24.53±9.21	<0.0001
Anterior layer (Φ 2–6 mm)	19.44±6.32	22.53±6.92	23.90±8.12	22.06±7.31	<0.0001
Central layer (Φ 0–2 mm)	23.63±8.75	28.56±9.98	27.77±9.46	26.11±9.20	<0.0001
Central layer (Φ 2–6 mm)	20.13±6.06	23.17±7.43	23.57±7.19	21.75±7.00	<0.0001
Posterior layer (Φ 0–2 mm)	23.31±8.60	26.64±9.53	30.63±13.51	29.23±12.45	<0.0001
Posterior layer (Φ 2–6 mm)	18.52±6.94	21.44±6.96	22.37±7.44	22.06±7.22	<0.0001
Total layer (Φ 0–2 mm)	27.42±9.64	31.55±10.04	35.84±13.33	29.27±10.68	0.002
Total layer (Φ 2–6 mm)	21.78±5.97	27.07±16.96	26.39±7.88	22.00±5.68	0.993

= Preoperation; (2) = 3 month postoperation; (3) = 6 months postoperation; (4) = 12 months postoperation.
Φ = annulus.

The mean corneal densitometry in the total preoperative layer was 19.10 ± 5.58, which changed to 22.31 ± 5.64 in the third month, 24.28 ± 6.37 in the 6th month and to 20.89 ± 5.73 in the 12th month, and these changes were significant in all areas. (P-value <0.05). The highest rate of changes occurred in the anterior layer and over the annular diameters 0-2 mm (from 22.38 ± 8.38 before surgery to 27.46 ± 9.63 in 3 month and 28.07 ± 11.28 in 6 month and 24.53 ± 9.21 in 12 month (P-value < 0.05)

The mean densitometry values peaked at 3 month (preoperative vs 3-month post hoc P = 0.006) and remained unchanged at 6 months (3-month vs. 6-month post hoc P = 1). It decreased significantly after 6 months (6-month vs. 12-month post hoc P = 0.001) and returned to almost preoperative values at 12 months.

The results of measuring corneal pachymetry before and after cross-linking are presented in Table 2.

Table 2. Changes in corneal pachymetry Values of Each Position of a Cornea before & after CXL(µm)

Variables	Preoperation (1)	3 month Postoperation (2)	6 months Postoperation (3)	12 months Postoperation (4)	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Thinnest location	426.17 ± 34.7	412.47 ± 42.58	-	-	0.005*
	426.17 ± 34.7	-	419.21 ± 37.3	-	0.025*
	426.17 ± 34.7	-	-	428.34 ± 36.9	0.392
*A significant difference was detected.					

The mean pachymetry of patients in corneal Thinnest location changed from 426.2 ± 34.7µm before surgery to 412.5 ± 42.6 µm in 3 month and 419.2 ± 37.3 µm in 6 month and 428.3 ± 36.9 µm in 12 month which was not statistically significant (P-value >0.05).

The results of measuring best corrected visual acuity (BCVA) before and after cross-linking are presented in Table 3.

Table 3. Changes in best corrected visual acuity (BCVA) before & after CXL(µm)

Variables	Preoperation (1)	3 month Postoperation (2)	6 months Postoperation (3)	12 months Postoperation (4)	P
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
BCVA Log Mar	0.10 ± 0.19	0.17 ± 0.20	0.059 ± 0.176	0.019 ± 0.15	<0.0001

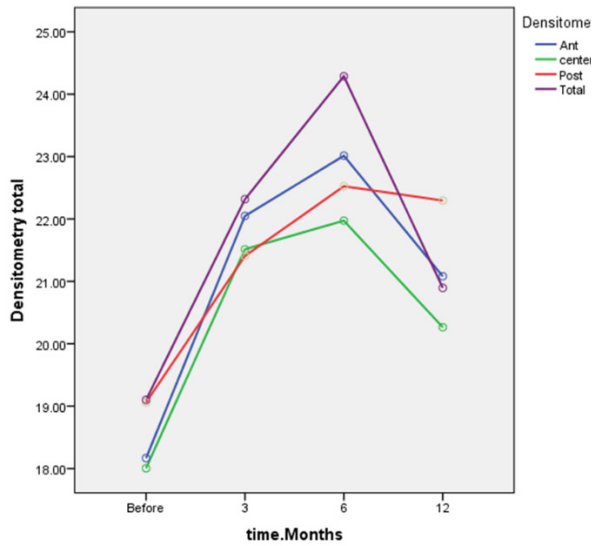

Figure 1. Comparison diagram of corneal densitometric changes in different layers and times before and after surgery

Visual acuity showed significant changes at different times before and after CXL (P <0.0001). Visual acuity decreased at 3 months after CXL compared to preoperative values (P <0.0001) and then increased in 12 months after CXL compared to preoperative values (P <0.0001).

Pearson correlation coefficient was used to measure the relationship between BCVA and densitometry before and 3,6,12 months after surgery, but the relationship was not significant. (p-value > 0.299)

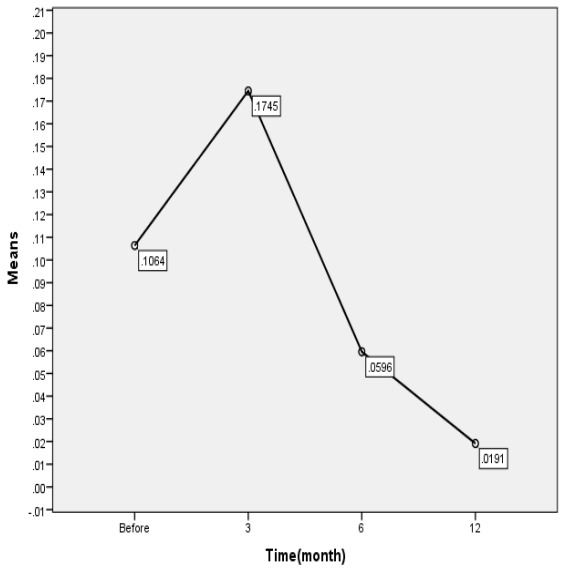

Figure 2. Comparison of BCVA-LogMar means at pre- and postoperative times.

4. Discussion

Discovering therapies is a challenge for many researchers and many studies are underway to find a highly reliable modality for the treatment of keratoconus. Due to the importance of collagen cross-linking in patients with keratoconus, the aim of this study is to comparison of corneal densitometry measured with Pentacam before and after collagen cross-linking [11].

The present study showed changes in corneal total densitometry during the first three months increased from 19.10 ± 5.5 to 22.31 ± 5.64 and then gradually returned to baseline values by 12 months (20.89± 5.73). These changes were greater in the 0-2mm center of the cornea and the anterior layer. In the study, Myriam Böhm showed a significant height of 3-month postoperative densitometry (P=0.015) relative to the baseline in the anterior stromal layer (120 µm) in concentric areas of 0.0 to 2.0 mm and 2.0 to 6.0 mm. A stromal demarcation line was evident at a depth of 203.00 µm ± 13.53 ± (SD). After CXL, no change in visual acuity of the modified distance (LogMAR) was observed, corneal thinning was measured with a significant reduction in central pachymetry (µm). In the present study the densitometry values of the anterior layer, the central layer, the posterior layer, and the total layer at 0 to 2 mm central corneal diameter and the values obtained at 2 to 6 mm central corneal diameter changed significantly after the CXL procedure.

Results showed that CXL increased corneal densitometry, especially in the anterior stromal layer in the two central concentric regions (0.0 to 2.0 mm and 2.0 to 6.0 mm) of the cornea at 3 months postoperatively. Changes in corneal densitometry in the anterior stromal layer were not related with postoperative visual acuity changes [11]. In the present study, there was a significant decrease in BCVA 3 month after CXL and then increase during 12 months after that. (BCVA log mar from 0.10 ± 0.19 to 0.019. 0.15 and P <0.05). There was no significant relationship between densitometry changes and changes in visual acuity in the postoperative months.

In the study of Picher et al., the mean densitometry of different layers of the cornea in 3 different areas increased 3 months after surgery. The mean densitometry in the region increased significantly from 0.0 to 2.0 mm after 12 months. The mean cross-section peaked after 1 month and then steadily decreased but remained high after one year. [12].

In a study by Semra Akkaya Turhan in Turkey, which was performed to compare changes in corneal density after two different cross-linking protocols. The corneal density in both groups reached a peak within one month after surgery and remained constant for up to 6 months and then continued to decrease for up to 12 months to return to baseline values [13].

In the present study, the examination of both eyes was significant in terms of visual acuity after CXL and during 12 months.

In Haney Helaly's study, the mean corneal densitometry was initially 16.30 ± 1.90 (measured on a 100 scale). In 1 month after surgery, corneal densitometry increased to 28.881 ± 4.33. Between 6 and 12 months after surgery, the corneal densitometry decreased by 4.78, which was statistically significant (P <0.001). At 12 months postoperatively, corneal densitometry did not return to baseline [14].

Several studies have attempted to explain the possible changes and mechanisms leading to CXL-related corneal opacity. Mazzotta et al.[15] examined corneal ultrastructural changes in KC eyes under CXL using Heidelberg retinal tomography II microscopic microscope. They found that corneal stroma was attacked by activated keratocytes, which was almost complete at 6 months. Active keratocytes have changes in their crystalline proteins that increase light scattering and possibly increase turbidity. A period of increased postoperative blurring at 1 month, followed by a gradual decrease, but no return to baseline at 1 year was also in line with the reported by Gutiirrrez et al.

In the present study, BCVA decreased during the 3 months after surgery, which was not found to be related to densitometric changes. BCVA values have changed significantly over the past 12 months compared to preoperative values. No correlation was found between changes in BCVA and

densitometric changes in the postoperative months.

5. Conclusion

According to these results, use of CXL can increase densitometry during the three-month postoperative and is effective in the patient's BCVA. The highest rate of changes occurred in the anterior layer and over the annular diameters 0-2 mm. No association was found between densitometric changes and changes in visual acuity. CXL can reduce corneal thickness in thinnest location during the three-month postoperative period which subsequently increases in next 12 months after surgery.

Densitometric values and corneal thickness return to baseline preoperative values to a large extent during 6-12 months after surgery. The present study also faced some limitations, including the fact that this study was conducted with a relatively small number of patients. Other limitations of the present study are: lack of sensitivity to it, lack of side effects and type of complication and severity of symptoms.

It is recommended that a study be performed in patients with keratoconus at different stages of the disease who have undergone CXL and that densitometric changes and corneal thickness be evaluated for 24 months after surgery. It is also suggested that a study be designed to evaluate and compare densitometric changes and corneal thickness in a group of patients undergoing CXL and a group undergoing treatment other than CXL.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Lema I., Sobrino T., Duran J.A., Brea D. and Diez-Feijoo, E. 2009. Subclinical keratoconus and inflammatory molecules from tears. *British Journal of Ophthalmology*, *93*: 820-824.

2. Elbedewy H.A., Wasfy T.E., Soliman S.S., Sabry M.M., Awara A.M., El Emam S.Y., Shafik H.M. and Alam M.R. 2019. Prevalence and topographical characteristics of keratoconus in patients with refractive errors in the Egyptian delta. *International ophthalmology*, *39*: 1459-1465.

3. Lapeyre G., Fournie P., Vernet R., Roseng S., Malecaze F., Bouzigon E. and Touboul D. 2020. Keratoconus Prevalence in Families: A French Study. *Cornea*, *39*: 1473-1479.

4. Soiberman U., Foster J.W., Jun A.S. and Chakravarti S. 2017. Suppl-1, M9: Pathophysiology of Keratoconus: What Do We Know Today. *The open ophthalmology journal*, *11*, p.252.

5. Espandar L. and Meyer J. 2010. Keratoconus: overview and update on treatment. *Middle East African journal of ophthalmology*, *17*: 15.

6. Pron G., Ieraci L., Kaulback K., Secretariat M.A. and Health Quality Ontario, 2011. Collagen cross-linking using riboflavin and ultraviolet-a for corneal thinning disorders: an evidence-based analysis. *Ontario health technology assessment series*, *11*: 1.

7. Hovakimyan M., Guthoff R.F. and Stachs O. 2012. Collagen cross-linking: current status and future directions. *Journal of ophthalmology*, *2012*.

8. O'Brart D.P. 2014. Corneal collagen cross-linking: a review. *Journal of optometry*, *7*: 113-124.

9. Peponis V., Kontomichos L., Chatziralli I., Kontadakis G. and Parikakis E. 2019. Late onset corneal haze after corneal cross-linking for progressive keratoconus. *American journal of ophthalmology case reports*, *14*: 64-66.

10. Dhawan S., Rao K. and Natrajan S. 2011. Complications of corneal collagen cross-linking. *Journal of ophthalmology*, *2011*.

11. Böhm M., Shajari M., Remy M. and Kohnen T. 2019. Corneal densitometry after accelerated corneal collagen cross-linking in progressive keratoconus. *International ophthalmology*, *39*: 765-775.

12. Pircher N., Pachala M., Prager F., Pieh S. and Schmidinger G. 2015. Changes in straylight and densitometry values after corneal collagen crosslinking. *Journal of Cataract & Refractive Surgery*, *41*:1038-1043.

13. Turhan S.A. and Toker E. 2017. Changes in corneal density after accelerated corneal collagen cross-linking with different irradiation intensities and energy exposures: 1-year follow-up. *Cornea*, *36*: 1331-1335.

14. Alzahrani K., Cristian C., Harper S., Carley F., Brahma A., Morley D. and Hillarby M.C., 2018. Corneal imaging and densitometry measurements in keratoconus patients to monitor disease progression and treatment outcomes after contact lens or intacs treatment. *Clinical Ophthalmology (Auckland, NZ)*, *12*: 1653.

15. Mazzotta C., Balestrazzi A., Traversi C., Baiocchi, S., Caporossi T., Tommasi C. and Caporossi A., 2007. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructural analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. *Cornea*, *26*: .390-397.