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RESEARCH ARTICLE

Evaluation of corneal endothelial cell changes following modified corneal collagen accelerated pulsed cross-linking in moderate keratoconus

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ABSTRACT

Purpose: The main aim of this study was to evaluate corneal keratometric and endothelial cell changes after a modified corneal collagen accelerated cross-linking with vitamin B2 (Riboflavin) for 10 minutes, and accelerated UVA irradiation, for the treatment of Keratoconus. Customized delivery of UVA radiation exposure limited to 18mw/cm2 instead of 30 mw/cm2 over 6 minutes.

Methods: Twenty keratoconus eyes, followed up for 6 months after epithelium-off accelerated CXL crosslinking with fixed 10 minutes riboflavin soaking and only 18 mw/cm2 ultraviolet exposure for 6min. Pentacam HR was used for measuring steep & flat anterior keratometry, Kmax and corneal thickness pre. & post operatively. Visual & specular microscopic changes were studied before and 6 weeks after CXL for observing endothelial cell changes after the procedure.

Results: Keratometric values showed significant reduction in corneal steepening. Kmax showed a mean reduction of 3.754 D with a remarkable significance p <0.0001. On the other hand the UCVA and BCVA showed insignificant change where as, the spherical equivalent significantly reduced by 3.666 and p <0.0001. Specular study revealed non-significant changes in the mean cell density values p= 0.940. The average mean AVG, also showed non significant changes p= 0.856. The mean percentage of hexagonality significantly decreased (-2.806) and accordingly the mean CV% by (–2.392) p=0.005 and p = 0.17 respectively. Standard deviation values SD, also deceased by – 3.920 d p<0.0001.

Conclusion: CXL safety is recommended as it is becoming the standard treatment caption for progressive Keratoconus world wide. We strongly recommend that the irradiance level should be clearly minimized to the level which induces therapeutic goal and low toxic effects.

Keywords: Keratoconus - Specular microscopy - Cross-linking.

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Introduction

Keratoconus is an evolutive bilateral progressive non-inflammatory ectatic disease of the cornea with onset generally at puberty[1].

Pathologically changes in corneal collagen structure and intercellular matrix, as well as apoptosis and necrosis of keratocytes, exclusively involving the central anterior stroma and the Bowman's membrane are documented in structurally weakened corneal tissue typical of keratoconus[2,3]. Corneal Collagen Cross-linking, CXL is one of the first treatments for Keratoconus that addresses the underlying cause of corneal weakening thereby stabilizes stromal collagen, increasing the biomechanical stability of the cornea. However, standard CXL (SCXL) protocol, with total treatment times in excess of 1 hour, researchers have proposed accelerated CXL (ACXL) protocols, to improve convenience and comfort for patients. These ACXL protocols have the aim of decreasing UVA exposure time by increasing UVA fluency to achieve the same overall total UVA dosage[4,5].

According to the rule of Bunsen and Roscoe, a photochemical reaction is directly proportional to the total energy dose, irrespective of the time over which this dose is delivered. At present the ACXL protocols are carried out in a shorter period such as 3, 5, or 10 minutes by using 30, 18, or 9 mW/cm2 irradiance, respectively, with a cumulative irradiation dose of 5.4 J/cm^2. The shorter corneal exposure time of ACXL, it has been proposed, might have the potential advantages of reducing the rate of complications such as corneal thinning, haze, infection, and melting[6].

There is a growing concern about the effect of this treatment on corneal endothelium[7]. 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[8].

As the cytotoxic level of endothelial UVA irradiance exceeds 0.35 mW/cm^2, which is, approximately twice the UVA reaches the corneal endothelium in standard protocol (0.18 W/cm^2)[9].

Patients and Methods

This study was a prospective, non randomized, clinically controlled, case series of 20 eyes, belonging to eleven patient, 16 females (80%) and 4 males (20%) with equal laterality. All patients diagnosed as progressive Keratoconus grade II at the Research Institute of Ophthalmology, Beni-Suef University, Ophthalmology dept. of Al-Rowad Eye hospital (Lasik Centre), Dokki. Cairo.

Acceptance criteria were strictly followed as progressive Keratoconus, contact lens intolerance, and a clear cornea as well as exclusion criteria were applied to those with surgical contraindications.

All the patients, completely examined preoperatively for UCVA, BCVA, Corneal topography and pachymetry using the Pentacam &Specular microscopic study on Nidek non contact specular microscope.

Informed consent was obtained from all patients before study enrollment. All patients were informed about the potential hazards of the surgery and the postoperative complications reported in the literature.

Surgical procedure: The procedure details was as written in any publication[10]. Epithelium scraping using a hokey blunt spatula, leaving the Bowman's layer clean and shiny and excluding post operative complications of alcohol. Riboflavin 0.1% solution (vibex-rapid) by Avedro installed for 10 complete soaking minutes.

The UVA-irradiation delivered using the KXLTM system (Avedro, Walthan, Massachusetts, USA) for 6 minutes with a pulsed pattern and a light intensity of 18 mW/cm^2 and a total delivery dose of 7.2 g/cm^2. Oxygen delivery tube fixed beside the cornea to ensure continuous photo-chemical reaction.

Contact lenses were applied and left until complete epithelial healing 4-5 days later with daily drops tobramycin & dexamethasone t.d.s. for two weeks. Slit lamp examination & visual record every 2 weeks, while corneal topography & specular microscopy performed at the end of 6th week, to ensure clear media & trustable values.

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Pre-coded data was loaded on a computer "Microsoft Office Excell Software" program in the form of pre- and post-operative visual, topographic & specular microscopic endothelial cell values. Data was then transferred to the statistical package program (Wilcoxon Signed Ranks Test) P-values equal to or less than 0.05 were considered statistically significant.

Data includes UCVA & BCVA, Refraction Spherical Equivalent, Kmax Topographic Central Corneal Thickness (CCT), Thinnest location value (TL) and KC grade. Specular microscopy values included pre- and post-operative, number of cells (NM), Cell density (CD), Average area (AVG), Standard deviation (SD), Coefficient of variation(CV), Hexagonal cells percentage(HEX) and corneal thickness (CT).

Results

We performed a prospective clinically controlled comparative study on 20 keratoconic eyes , belonging to 11patients, 16 females (80%) and 4 males (20%) with equal laterality (figure 1 - tables 1a&b). All patients were grade II Keratoconus and completed one and half month follow-up after treatment where visual stability was expected.

As regards UCVA detailed results shows no significant changes between pre- and post-operative values with $p>0.5$ in both examinations. The, BCVA remained unchanged by the end of the postoperative period with $p=0$. On the contrary, a significant decrease in spherical equivalent postoperatively when compared to preoperative values $p<0.05$. (tables 2,3,4)

Keratometric values showed significant reduction in corneal steepening. Improvement of the mean minimum and maximum kmax readings achieved with $p=0.001$. (table 4).

Pachymetric thinnest location (PL) in Penta-Cam examination is a very sensitive parameter in postoperative edema detection and treatment evaluation. A significant thinning of treated corneas is achieved, $p=0.009$. (table 2,3, 4,5,)

		Count	Column N %
Gender	F	16	80.0%
	M	4	20.0%
ODOS	OD	10	50.0%
	OS	10	50.0%
KC grade	II	20	100.0%

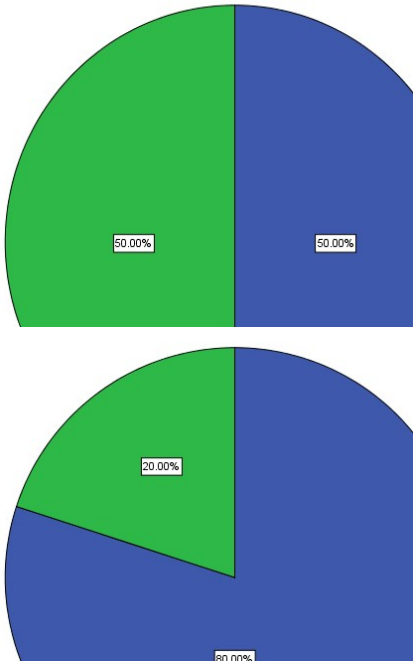

Table (1a), figure (1): General patient's data, gender, OD/OS, & KC grade.

	Mean	Standard Deviation	Median	Minimum	Maximum
Age	**23**	**7**	**22**	**15**	**35**

Table (1b): patient's age data

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	Mean	Standard Deviatio	Median	Minimum	Maximum
Age	23	7	22	15	35
Pre Kmax	51	1	52	49	52
Pre T.L	446	27	447	400	488
Pre UCVA	.5	.1	.5	.1	.6
Pre BCVA	.7	.1	.7	.5	.9
Pre SE	-3	2	-3	-6	1
Pre NC	172	67	167	51	278
Pre CD	2658	652	2850	291	3382
Pre AVG	368	49	351	296	467
Pre SD	85	22	78	64	143
Pre CV	24	4	23	19	33
Pre HEX	71	5	71	59	78
Pre CCT	460	23	462	410	499

Table (2): Pre-operative data with detailed analysis. Kmax =maximum k value, TL= thinnest location, SE= spherical equivalent, NC=number of cells, CD= cell density cell/mm, AVG= average area um2, SD= standard deviation um2, CV= coefficient of variation%, HEX= hexagonal cells %, CCT= central corneal thickness

	Mean	Standard Deviation	Median	Minimum	Maximum
Post Kmax	48	2	48	45	51
Post T.L	435	36	438	359	490
Post UCVA	.4	.1	.5	.3	.6
Post BCVA	.7	.1	.7	.5	.9
Post SE	-2.8	1.8	-2.6	-5.5	1.0
Post NC	130	67	125	40	276
Post CD	2761	322	2885	2187	3217
Post AVG	364	43	347	311	457
Post SD	91	23	88	64	143
Post CV	26	5	25	19	34
Post HEX	66	6	67	55	76
Post CCT	451	28	452	400	497

Table (3): post-operative statistical values with detailed analysis

	Post Kmax – pre Kmax	Post T.L preT.L	Post CT – pre CT	Post UCVA – pre UCVA	Post BCVA – pre BCVA	Post SE – pre SE
Z	-3.754b	-3.194b	-2.882b	-.535b	.000c	-3.666d
Asymp. Sig. (2-tailed)	.001	.009	.004	.593	1.000	.000

Table(4): significance values visual and keratometric

The mean value of number of cells (NO) showed a significant reduction, p=0.001.The NO are always variable and the probability is low in repeated examinations. (table 5). The mean value of endothelial cell density, ECD, shows no statistical significance, p =0.4. Also the average cell area AVG, showed non significant changes in cross linked corneas (p= 0.6). (table 5). The coefficient of cell variation CV, which is the percentage of calculated polymegathism, showed preoperative mean value 24+/-4 % , and postoperative 26 +/-5 %, with a statistical significance (p=0.015). (tables 3,5).

	Post NO Pre NO	Post CD pre CD	Post AVG pre AVG	Post SD pre SD	Post CV pre CV	Post HEX pre HEX. Cells
Z	-3.436b	-.075b	-.181b	-3.920d	-2.392d	-2.806b
Asymp. Sig. (2-tailed)	.001	.940	.856	.000	.017	.005
a. Wilcoxon Signed Ranks Test						
b. Based on positive ranks.						
c. The sum of negative ranks equals the sum of positive ranks.						
d. Based on negative ranks.						

Table 5. Significance specular microscopic values

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The mean percentage of hexagonal cells in specular charts was 71% +/- 5 preoperatively, postoperatively 66% +/- 6, and a high significant value of p=0.003. Also standard deviation SD values, proved statistical significance p=0.05 (tables 3,5). As regard central corneal thickness CCT, the mean value dropped and showed a statistical significance p=0.006. (tables 2,3,4,5,)

Discussion

CXL is a photo-polymerization increases the rigidity of corneal collagen. The aim is to slow or arrest progression to delay or avoid Keratoplasty[11].

There is a growing concern about the effect of CXL treatment on corneal endothelium[7]. In this study we have chosen a new customized treatment in which only 18 mw/cm^2 delivered in a pulsating mode for six minutes pulsed exposure time, to avoid endothelial cell damage. Soaking time was kept constant as recommended, 10 minutes, for all cases.

In our study the Kmax showed a mean reduction of 3.754 D with a remarkable significance <0.0001. On the other hand the UCVA and BCVA showed insignificant change where as, the spherical equivalent significantly reduced by 3.666 and p <0.0001.

Shajari et al have compared conventional and accelerated CXL and found a significant difference in the D value (a value that involves anterior and posterior corneal elevation, corneal pachymetry, and progression of corneal thinning) with more favorable results for the conventional procedure. However, other studies have demonstrated that both procedures have a comparable effect in stabilizing keratometry[12].

In this study, there was non significant changes in the mean cell density values p= 0.940. The average mean AVG, also showed non significant changes p= 0.856.

The mean percentage of hexagonality significantly decreased (-2.806 b) and accordingly the mean CV% decreased by –2.392 d p=0.005 and p = 0.17 respectively.

Standard deviation values SD, also deceased by – 3.920 d p<0.0001 which positively adds to the previous results.

Several studies reported on endothelial cell counts after cross-linking. Some of them reported no change in endothelial cell counts, whereas others reported reduction in endothelial cell counts and the remaining reported a small increase in endothelial cell counts.

Hasan Razwjoo et al reported there was a significant reduction in ECD after CXL (p-value = 0.004). The least decrease in cell density was found in patients with corneal thickness less than 450 µm, while these patients had the thinnest cornea. The mean $\pm$ SD of the coefficient of variations of the endothelial cell size (CV) before and after the CXL were 32.72 $\pm$ 10.14 and 40.21 $\pm$ 9.70, respectively (p-value = 0.021)[13].

The mean $\pm$ SD of the preoperative percent of hexagonal cells (pleomorphism) was 54.14 $\pm$ 6, and the postoperative percent was 54.55 $\pm$ 5 (p-value = 0.517) indicating non significant changes.

Goldich et al showed stable ECD in 14 keratoconic eyes during the early and the late periods following the UVA/riboflavin CXL treatment. Similar stability of ECD was reported in other preliminary results of randomized clinical trial[14].

In accordance with these studies, there was no significant change in corneal endothelium postoperatively when compared with preoperative levels in the treated eyes, which was suggested to be the result of the profile of our treatment.

Ucakhan et al observed some changes in both density and the morphology of endothelial cells that become more prominent with the increasing grades of the disease. They proposed that in KC, unstable endothelium becomes susceptible to damage, and thus endothelial damage following CXL may be related to an indirect response of this unstable endothelium against UVA irradiation and consequent oxidative stress[15].

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Regarding CCT, our study reported significant decrease postoperatively in comparison to preoperative values, p=0.004. These results were similar to pentacam maps measuring the thinnest corneal location p <0.0001.

Several studies reported data on pachymetry. Some of them reported reduction in pachymetry (median change: 11.33µm, range 66.46-0.24µm), others reported no change and the remaining studies showed increase in pachymetry (median change: 4.63 mm, range 0.6-37 µm) [12,15,16].

Elsaftawy et al reported that at one month postoperatively, pachymetry at the thinnest location showed thinning by 38.69 µm (p-value <0.000) [16].

Hasan Razmjoo et al reported that: mean ± SD of preoperative and postoperative pachymetric values were 470 ± 40 µm and 469.8 ± 42 µm, respectively (p-value = 0.591) [13].

CCT measured by specular microscopy is believed to be inaccurate compared to pachymetry. Mild corneal edema and ongoing healing process in the corneal epithelium may interfere with the specular microscopic measurements[14].

Nevertheless we presented pachymetry measurements in addition, for more confirmation.

Conclusion

CXL safety is recommended as it is gradually becoming the standard treatment caption for progressive Keratoconus world wide.

The photo chemically induced free radicals through the UVA irradiation on a sensitized cornea have itself the potential to induce endothelial damage.

We strongly recommend that the irradiance level and the soaking period of time should be clearly minimized to the level which induces therapeutic goal and low toxic effects. Speculator microscopic evaluation of corneal endothelium should be documented before and after CXL treatment.

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