ORIGINAL ARTICLE

SAFETY OF HIGH INTENSITY ACCELERATED CORNEAL COLLAGEN CROSS-LINKING IN KERATOCONUS PATIENTS ON BASIS OF ENDOTHELIAL CELL DENSITY

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Background: This study was conducted to evaluate the safety of accelerated corneal collagen crosslinking (CXL) in keratoconus patients on basis of endothelial cell density measurements. **Methods:** We studied 24 patients (42 eyes) with diagnosed keratoconus who underwent accelerated CXL with 9 Mw/cm^2 UVA irradiance for 10minutes. All patients underwent detailed examination and video keratography (VKG) for classification and confirmation of keratoconus. Patients with central corneal thickness (CCT) of ≥400µ were included in the study. Specular microscopy was done to note endothelial cell density (ECD) preoperatively and then at the end of 1st, 4th and 12th weeks' post-operative period. **Results:** The study included 24 patients (42 eyes) from October 2016 to June 2017. Among these 13 were females and 11 males with mean age of 20.15±6.73 years. Eighteen patients underwent the procedure in both eyes while 6 had the procedure in one eye. The pre-operative ECD mean±SD of right eye was 2743.97±542.77/mm^2 and left eye was 2763.35±532.57/mm^2. The post-operative ECD mean±SD of right and left eyes at the end of 12th post-op weak were 2806.34±520.11/mm^2 and 2823.30±628.57/mm^2 respectively. The pre and post-op ECD comparison showed p-values at first week post-op are 0.474 and 0.683 for right and left eyes respectively. Similarly, the *p*-values at 4th and 12 weeks post-op for right eye are 0.266 and 0.280 respectively. The *p*-values at 4th and 12th weeks for left eye are 0.913 and 0.404 respectively. **Conclusion:** Accelerated CXL protocols is safe and effective procedure and did not lead to significant change in ECD in our study population in three months post-procedural follow up. However further research is required to determine the effect of high intensity UVA radiation on other ocular structures with larger group of patients and long-term follow up.

Keywords: Keratoconus; Prevalence; Epithelium-off corneal crosslinking

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INTRODUCTION

Keratoconus is a non-inflammatory disorder[1] in which cornea assumes a conical shape secondary to stromal thinning and progressive protrusion leading to irregular astigmatism. Both eyes are affected, at least on topographical imaging, in almost all cases.[2] The worldwide reported prevalence of keratoconus varies widely depending upon the geographic location, Environmental, ethnic factors, diagnostic criteria used and the cohort of patients selected.

Visual deterioration occurs with progressive corneal thinning and steepening that leads to irregular astigmatism.[3] In the early stages glasses and rigid gas permeable contact lenses might help, however, progressive nature of disease renders those insufficient and patient might need a surgical alternative such as intra-corneal ring segments, corneal collagen crosslinking (CXL) or corneal transplant.[4,5]

Corneal collagen crosslinking is a negligibly invasive surgical technique used to halt the progression of keratoconus. This technique involves the use of riboflavin also known as vitamin B2, in combination with ultraviolent-A irradiation which results in the formation of chemical bonds between collagen fibrils in corneal stroma.[6] Thereby, CXL strengthens the cornea and slows or might even stop the progression of keratoconus.[7] The efficacy and safety of standard Dresden protocol has been reported in several studies since its inception in 1998. In their clinical study Wollensak *et al*[8] showed the efficiency and safety of standard Dresden protocol with follow-up of up to four years. In the Dresden protocol, after epithelial debridement and riboflavin saturation of stroma is done for 30 minutes, UVA light is then used for illumination at the intensity of 3 mW/cm^2 for 30 minutes. This delivers a total of 5.4 J/cm^2 energy to the cornea.[9] To shorten the treatment span of CXL procedure and improving patient comfort and cooperation, an increase in illumination intensity has been used. The concept is based on "Bunsen-Roscoe law of reciprocity" which states that effect

of photochemical reaction is similar if the intensity and time is changed keeping the total energy delivered the same.[10] Thus, the total energy delivered (5.4 J/cm^2) in standard CXL should be similar to irradiation at 9 mW/cm^2 for 10 minutes.[10]

This study is conducted to evaluate the safety of accelerated CXL by noting the effect of high intensity UVA radiation on endothelial cells density. The rationale of our study is to gather more evidence about safety of accelerated CXL treatment (9 mW/cm^2 for 10minutes). The objective of the study was to determine the safety in terms of CCT & ECD of accelerated corneal collagen cross linking in the treatment of keratoconus.

MATERIAL AND METHODS

The Quasi-experimental study was conducted Armed Forces Institute of Ophthalmology (AFIO), affiliated to National University of Medical Sciences (NUMS) Rawalpindi from Nov 2016 to July 2017. Forty-two eyes of 24 patients were selected through non-probability consecutive sampling.

Inclusion Criteria

- We included patients between the ages of 9-40 years irrespective of gender
- Corneal thickness ≥ 400 µm

Exclusion criteria:

Patients with keratoconus having any of the following;

1. Central or paracentral corneal opacity
2. Active ocular infection
3. Severe keratoconus
4. Pregnancy and lactation
5. Autoimmune or collagen disease

Data was collected for demographic variable like age in years and gender and research variable like CCT and ECD. After roper clinical history, clinical and ophthalmological examination and required laboratory test; diagnosis was made. Required surgical procedure was explained to each patient and written consent was taken as required by hospital ethical committee. Endothelial cell density was taken using specular microscopy (SP-3000P) and readings interpreted by attached computer loaded with dedicated software.

The cell density was calculated as number of cells per mm^2 (No of cells/mm^2). Sample of such counting (attached as Figure-1.) was done before the surgery. After the surgery bandage contact lenses (BCL) were applied and follow up was performed at 1, 4 and 12 weeks and specular microscopy was performed at each follow up visit. BCL was removed after one week. No intraoperative or postoperative complications were noted in our study.

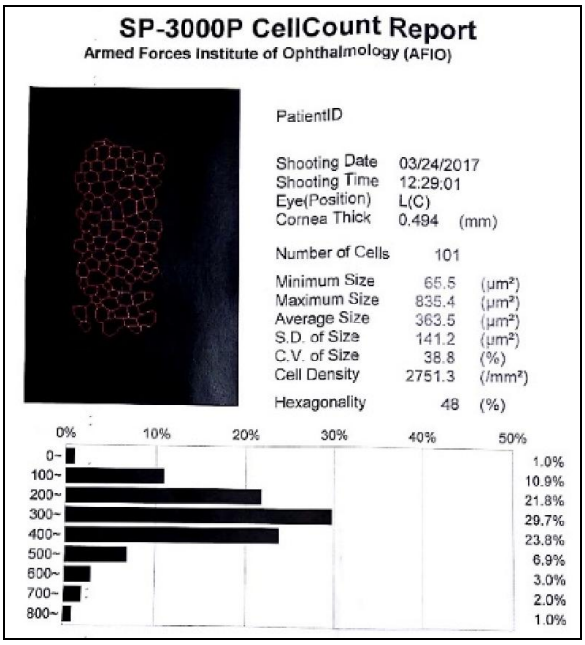

Figure-1: Sample of SP-3000P endothelia cell density report

Procedure was performed by same surgeon under strictly aseptic conditions. Topical anesthetic drops proparacaine HCL 0.5% (alcaine by Alcon Pharma Karachi) were instilled in the eye. Patients were placed in the supine position and lid speculum was placed. Central 8 mm of corneal epithelium was removed with kimura spatula. After removal of epithelium 0.1% riboflavin solution (riboflavin-5-phosphate in dextran) was instilled at the frequency of 2–3 minutes for 30 minutes. After that 9mW/cm^2 UVA radiation was applied by using CCL-VARIO corneal cross-linking system Swiss med Asia Singapore for 10 minutes, so that the total energy given was 5.4 J/cm^2. Riboflavin eye drops were instilled every 5 minutes during UVA radiation to keep the cornea saturated.

After the procedure hydrogel bandage contact lens were applied to allow re-epithelization. Topical vigamox (0.5 % moxifloxacin) eye drops qid and Systane (Polyethylene Glycol 0.4% + Propylene Glycol 0.3%) 2 hourly were prescribed. Patients were then followed up at the end of one, four and twelve weeks post operatively. Eye examination and ECD was measured on each follow-up.

Statistical analysis was performed using the SPSS-16.0. Descriptive statistics were used to describe this information and data. Chi square test was applied to compare qualitative variables between the pre-and post-procedural changes. Independent samples' t-test was used to compare pre-and post-procedural cell density and corneal thickness of each eye. Continuous and categorical variables are

reported as mean±SD and percentages, respectively. A *p*-value <0.05 was considered as significant.

RESULTS

Twenty-four patients, 11 males and 13 females (42 eyes, 21 right and 21 left eyes) were included in the study with age ranging from 9–40 years. Mean age of the patients was 20.15±6.73 years. The mean central corneal thickness of each eye summarized in table-1. The pre-and post-procedural (1st, 4th and 12th post procedural weeks) mean±SD Endothelial cell density for right and left eye summarized in table-2. In 18 patients, surgical procedure was performed on both eyes and in three patients' procedure was done on right and in remaining three it was performed on left eyes. Pre-versus post procedural ECD comparison at 1st, 4th and 12th weeks of right and left eyes are summarized in Table-3. The comparison was made between baseline ECD and post-procedural ECD together with post-procedural intergroup comparisons. The pre-operative ECD mean±SD of right eye was 2743.97±542.77/mm^2 and left eye was 2763.35±532.57/mm^2. The post-operative ECD mean±SD of right and left eyes at the end of 12th post-op weak were 2806.34±520.11/mm^2 and 2823.30±628.57/mm^2 respectively. There is no statistically significant difference in the *p*-value as compared to the baseline data. The pre and post-op ECD comparison showed *p*-values at first week post-op are 0.474 and 0.683 for right and left eyes respectively. Similarly, the p-values at 4th and 12 weeks post-op for right eye are 0.266 and 0.280 respectively. The *p*-values at 4th and 12th weeks for left eye are 0.913 and 0.404 respectively.

Table-1: Baseline mean±SD CCT

		Corneal Thickness for Right Eye	Corneal Thickness for Left Eye
n	Valid	21	21
	Missing	0	0
Mean		494.19	489.14
Std. Deviation		39.34	44.17

Table-2: The pre and post procedural mean±SD endothelial cell density (ECD)

		Pre-op RT Eye	Postop 1st week Rt eye	Postop 4th week Rt eye	Postop 12th week Rt eye	Pre-Op LT Eye	Postop 1st week Lt eye	Postop 4th week Lt eye	Postop 12th week Lt eye
N	Valid	21	21	21	21	21	21	21	21
	Missing	0	0	0	0	0	0	0	0
Mean		2743.97	2701.97	2814.82	2806.34	2763.35	2719.28	2770.93	2823.30
Median		2751.10	2633.71	2713.30	2788.92	2715.15	2711.30	2804.65	2837.40
Std. Dev		542.77	626.59	500.44	520.11	581.65	635.14	532.57	628.57
Minimum		1175.60	1172.00	2065.00	1981.80	1774.28	1003.70	1751.60	1568.80
Maximum		3882.20	3887.30	3892.40	3886.40	3884.60	3914.40	3684.60	3899.80

Table-3: Pre vs post-op Endothelial cell density

		Paired Differences					Sig. (2-tailed)
		Mean diff	SD	Std. Error Mean	95% Confi interval Lower	Upper	
Pair 1	preop_Rt_eye - postop_1_week_RT	42.00	282.54	57.67	-77.30	161.30	.474
Pair 2	preop_Rt_eye – vs postop_4_week_RT	-70.84	304.69	62.19	-199.50	57.80	.266
Pair 3	preop_Rt_eye – vs postop_12_week_RT	-62.36	276.29	56.39	-179.03	54.30	.280
Pair 4	postop_1_week_RT – vs postop_4_week_RT	-112.85	269.01	54.91	-226.44	.74	.051
Pair 5	postop_1_week_RT – vs postop_12_week_RT	-104.36	292.48	59.70	-227.80	19.13	.094
Pair 6	postop_4_week_RT vs postop_12_week_RT	8.48	152.67	31.16	-55.98	72.94	.788
Pair 7	preop_LT_eye – vs postop_1_week_LT	44.06	452.17	92.30	-146.87	235.00	.638
Pair 8	preop_LT_eye – vs postop_4_week_LT	-7.57	336.84	68.73	-149.81	134.65	.913
Pair 9	preop_LT_eye – vs postop_12_week_LT	-59.95	345.75	70.57	-205.94	86.04	.404
Pair 10	postop_1_week_LT – vs postop_4_week_LT	-51.64	306.84	62.63	-181.21	77.92	.418
Pair 11	postop_1_week_LT - postop_12_week_LT	-104.01	267.03	54.50	-216.77	8.74	.069
Pair 12	postop_4_week_LT - postop_12_week_LT	-52.37	216.05	44.10	-143.60	38.85	.247

DISCUSSION

Corneal collagen crosslinking (CXL) is now a days the first line treatment in progressive mild to moderate keratoconus[11] and Dresden protocol is standard method used for CXL. Standard Dresden protocol is proven safe by a number of studies. After the first in vivo study by Wollensak *et al*, a number of clinical studies have confirmed the safety of protocol and its role in halting the progression of disorder. Jankov *et al*[12]confirmed the safety and efficacy of the protocol in the conclusion of their study. Same trend was observed by Wittig-Silva *et al*[13], Mazzotta *et al*[14], Raiskup-Wolf[15] and Kymionis[16]clinical studies.

The limitation with Dresden protocol however; is about an hour-long procedure which becomes inconvenient for both patient and surgeon and only a limited number of procedures can be done

in a day. To solve the issue of time consumption, another approach was devised based on Bunsen-Roscoe law of reciprocity, which is to increase the amount of energy in a way that same amount of total energy could be provided in reduced amount of time and process of corneal collagen cross linking (CXL) could be accelerated. According to the law; if the total amount of energy is kept constant in less duration of time, same effect could be achieved and it can shorten the procedure timing and make it comfortable for both patient and surgeon. Some studies have described the limitations of the law that it is only true for radiations up to 45 mW/cm^2 and applied time more than 2 minutes.[17]

Since different structures in eye are very sensitive to UVA radiation and in higher intensities it is harmful for cornea, crystalline lens and retina, safety of the procedure must be ensured by repeat studies taking multiple factors in account. Many studies have taken multiple factors into consideration to establish the safety of accelerated CXL; Çınar *et al* compared the conventional and accelerated protocol in their study taking uncorrected and best corrected distant visual acuity, refractive errors, keratometry readings and ECD into account and concluded similar results at the end of 6 months.[18]similar results were reported by Elbaz *et al*[19] with 12 months follow up; Ulusoy *et al* at 12 months in pediatric patients[20] and Sherif *et al* in their clinical trial[21]. In local population study by Akbar B *et al* clinical outcome was described and showed almost similar results clinically without and measurement of ECD. However, they recommended the measurements of ECD[22].

In our study, ECD was taken as a measure of safety after the procedure, using Topcon SP-3000P specular microscope. We took the readings before and one week after the surgery. Follow up was performed at the end of 4 and 12 weeks respectively. We did not observe significant decrease in ECD by the end of 12 weeks. There was insignificant decrease in ECD at the end of first week in some patients, but it was progressively recovered on subsequent follow ups. This initial decrease can be attributed to early mild corneal oedema, but exact cause should be further studied.

Even though the results in our study complement other studies about safety of accelerated protocol, this study had a few limitations including, small number of patients, short term follow-up and taking two factors to evaluate the safety of the procedure. In that regard, more studies with large group of patients and long term follow up is required to ensure safety of the procedure.

CONCLUSION

Accelerated CXL protocols is safe and effective procedure and did not lead to significant change in ECD in our study population in three months post-procedural follow up. However further research is required to determine the effect of high intensity UVA radiation on other ocular structures with larger group of patients and long-term follow up.

Disclosure: No author has a financial or proprietary interest in any material or method mentioned, neither the article presented in any conference, seminar, and symposium before submission to J Ayub Med Coll Abbottabad.

Conflict of interest: This study has no conflict of interest to declare by any author.

AUTHORS' CONTRIBUTION

AF: Conceptualization of study, literature search, write-up, assistant surgeon, data collection. SH: Surgery. IF: Data collection. IB: Study design, proof reading. MI: Finalization with proof reading. FH: Data analysis and interpretation.

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