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Research Article

Evaluation of Crystalline Lens Density after Standard Corneal Collagen Cross-linking in Patients with Progressive Keratoconus

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Abstract

Purpose: To evaluate crystalline lens densitometry after Standard Collagen Cross-Linking (CXL) in patients with keratoconus (KCN).

Methods: In this cross-sectional, case-control study crystalline lens density was evaluated using the Pentacam (PNS system). Patients were investigated in the precisely before surgery, and then 3 and 6 months after CXL. Keratoconic patients were included in the study. CXL was done unilaterally and the fellow eye of these patients were considered as control group.

Results: The mean density of crystalline lens was 8.34% ± 0.67% prior to CXL in eye which undergone cross-linking and 8.34% ± 0.43% in the control group (P value: 0.59). Density of crystalline lens was evaluated 3 and 6 months after surgery. In eyes which undergone CXL, it was 8.40% ± 0.60% and 8.35% ± 0.84% and in the control eyes the evaluated density was 8.27% ± 0.51% and 8.33% ± 0.47%, which showed no significant difference (P-value was 0.179 and 0.51, respectively). Also, in eyes which undergone CXL crystalline lens density didn't differ significantly in the 3 and 6 months after surgery ($P > 0.05$).

Conclusions: Our study showed that there is no statistically significant difference in crystalline lens densitometry between the eyes which undergone CXL and naive eyes, before the procedure, 3 and 6 months follow up.

Keywords: *Corneal Collagen Cross Linking; Corneal Cross Linking; Keratoconus; Ultraviolet; Optical Coherence Tomography; Phototoxicity; Lens Densitometry*

Abbreviations

KCN: Keratoconus; CXL: Collagen Cross-Linking; UV-A: Ultraviolet-A; ROS: Reactive Oxygen Species; D: Diopter; OFR: Oxygen Free-Radicals; UV-B: Ultraviolet-B

Introduction

Keratoconus (KCN) is a corneal ectasia determined by irregular astigmatism and progressive visual loss due to corneal thinning and protrusion [1-2]. The definite pathology of KCN is unknown, but destruction and thinning of epithelial cell layer in primary stages introduced basal epithelial cell layer injuries as primary pathophysiology [3,4].

By performing collagen cross-linking (CXL), several covalent or ionic bonds are created which connect polymeric chains of collagen to each other and causes increase in endurance of collagen fibers [5]. This procedure is mediated by riboflavin in addition to Ultraviolet-A (UV-A) rays [6].

The standard treatment protocol of CXL is now referred to as the Dresden Protocol, and was initially described by Wollensak., *et al.* In this procedure the eye is anaesthetized and central 7 - 9 mm of the epithelium is removed. Then, riboflavin solution (0.1% riboflavin-5-phosphate and 20% dextran T-500) is applied to the corneal surface 30 minutes before irradiation and at 5 minutes intervals during the course of a 30 minute exposure to 370 nm UV-A with an irradiance of 3 $mWcm^{-2}$. Finally, antibiotic eye drops is instilled and a bandage contact lens is applied to the cornea [7].

There are several complications of CXL such as infectious keratitis [8-10], corneal edema and haziness [11,12], reduced corneal sensitivity [13,14], destruction of corneal limbal stem cells [15] and etc. In addition, some studies have showed complications of this procedure on intra-ocular tissues like transient retinal toxicity [16]. Unfortunately these studies about intra-ocular complications in this procedure are not conclusive.

Reactive oxygen species (ROS) cause oxidative damage to ocular tissues. Exogenous factors of ROS are UV light, visible light, ionizing radiation, chemotherapeutics, toxins and main endogenous factor is mitochondria. Because the crystalline lens cells and their intracellular proteins are not replaced over time it is highly susceptible to oxidative damage [17].

UV damages cell membranes of the lens and alters lens optics, which may subsequently lead to senile cataract formation [18]. This effect is thought to be due to the penetration of UV rays through the cornea and subsequent induction of photochemical generation of ROS and consequent oxidative stress in the aqueous and lens [19,20]. Although riboflavin acts as a light blocker during the procedure of CXL; but a small amount of radiation enters the eye and produce ROS [21]. Altogether, Few studies has shown that there are no lens density alteration after CXL treatment [22,23].

Pentacam, a scheimpflug imaging system, is an objective method to grade lens opacity [23]. This device creates a relatively reliable image of the anterior segment and shows a lens density at a scale of 0% to 100% [23-25]. Pentacam nucleus staging (PNS) is a lens density measurement program that shows the average and maximum density of lens [26].

In this study we evaluated the effect of CXL on lens density using pentacam scheimpflug imaging system.

Materials and Methods

This study was a cross-sectional observational study which was conducted from March 2016 and March 2017 at Nikookari Eye Hospital (Tabriz, Iran).

This cross sectional observational study reviewed the chart of patients examined at the ″Nikoukari Eye Hospital (Tabriz, Iran)″. It followed the Declaration of Helsinki principles and was approved by ″the ethics committee of the Tabriz University of medical sciences″. The confidentiality of the patient's data was secured all over the study.Before recruitment, the study design and its safety were explained for all of patients and informed consent was obtained.

Patients with bilateral KCN were referred to anterior segment clinic, was our population in this study and all of them were examined by an expert kerato-refractive surgeon (M.M.). Patients with progressive KCN were included in the study after providing informed consent. Progressive KCN was determined by following criteria in at least one eye: 1.increase in K max or corneal steep meridian more than 0.5 diopter (D) from baseline, 2. More than 20μm (micrometer) decrease in central corneal thickness, 3.incease in manifest cylinder more than 0.5D in 6 months.

27 eyes with progressive KCN from 27 patients were included as subject group and fellow eyes of these patient were included as control group.

All the patients with a previous ocular surgery, any corneal pathology (i.e. corneal scarring due to keratitis, dystrophy, trauma or hydrops), other ocular disorders (i.e. glaucoma, uveitis, cataract), systemic diseases with ocular involvement (i.e. connective tissue disease and diabetes mellitus) and pregnant patients at the time of the measurements, were excluded from the study.

Lens densitometry using the pentacam

In the Pentacam HR scheimpflug imaging (Oculus Inc., Germany), the camera captures 25 single slit images in less than 2 seconds with a rotation around the eye from 0 to 180 degrees and render a three dimensional (3D) representation of anterior segment. Other capability of this imaging is providing images of the crystalline lens and density analysis after pupil dilation [27]. Three sequential images of each eye were taken in a dark room.

Three disparate densitometric analyzes were carried out to detect crystalline lens density on a continuous scale (Figure 1): one 3-dimensional (3D) mode and two manual modes, i.e. the linear and region of interest (ROI) modes. These densitometric manner have been characterized in prior studies (24, 27). This software measures the crystalline lens density on a scale from 0-100% (0= no lens opacification; 100= totally lens opacity).

The linear mode measures the opacity of a vertical line manually drawn along the axial length of the lens. The ROI mode prepare a density quantity for a specific area drawn on an individual pentacam image. We manually drew an elliptical ROI focused on the center of the crystalline lens on the Scheimpflug image, eliminating its anterior and posterior cortex. For the 3D mode, the software of the Pentacam system (PNS software) automatically create a cylindrical schema for the density analyzes. The schema voluminosity used for this study had the following characteristics: diameter =4.0 mm, height =2.4 mm, front curvature =8.3 mm, and back curvature =4.8 mm. The 3D schema was focused in the crystalline lens center, with exclusion of the anterior and posterior cortical lens. The average and max density were observed for all densitometric state.

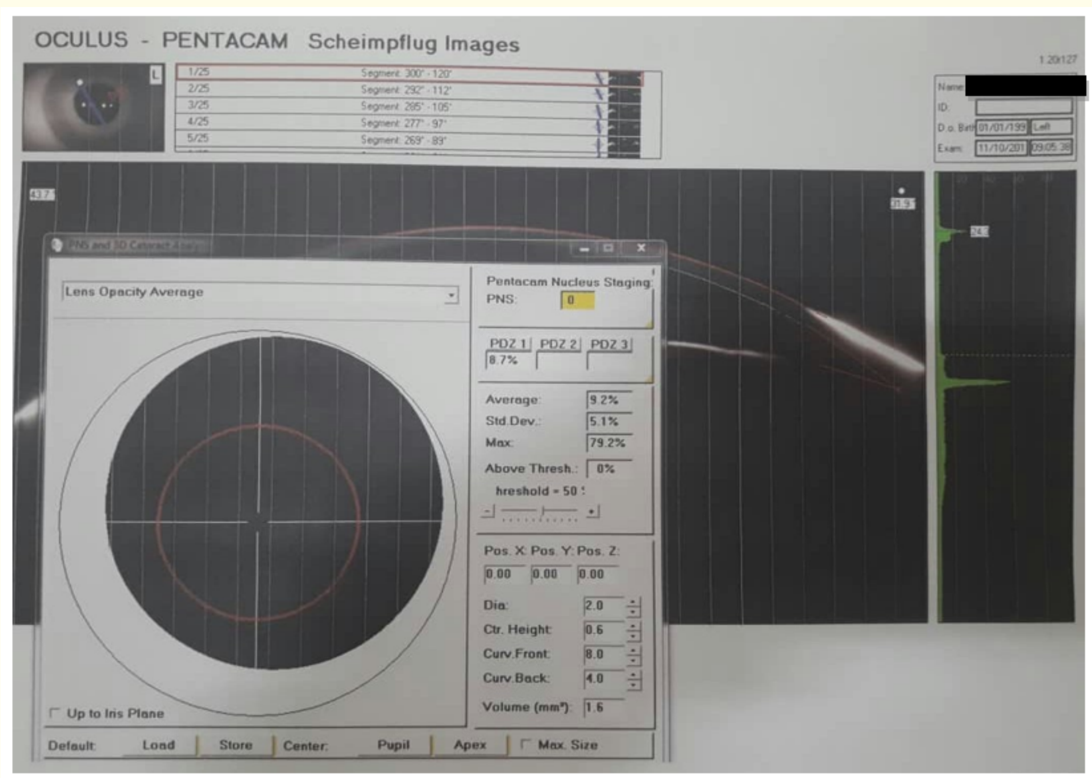

Figure 1: *PENTACAM Scheimpflug view of the crystalline lens densitometry in PNS system. (PNS: Pntacam Neuclear Staging)*

Statistical analysis

Data were analyzed using SPSS software for Windows (SPSS Inc., Chicago, IL, USA). In the statistical analysis independent-sample t test (used to compare quantitative data), chi-square test (used for qualitative data) . Normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were expressed as frequency and percentage for categorical variables whereas quantitative data were expressed as mean± standard deviation (SD) and median (minimum-maximum) for non-normally distributed data. A P<0.05 was considered statistically significant.

Results

54 eyes of 27 patients including 24 women (44.4%) and 30 men (55.6%) underwent standard CXL surgery for involved eye, and fellow eyes of them were included as controls. The mean age of patients was 21 years, and the minimum and maximum age of patients was 15 and 28 years, respectively. Twenty seven patients underwent CXL of the right eye and 26 patients of the left eye. The mean density of crystalline lens before surgery in the eyes underwent CXL was 8.34 ± 0.67% (minimum and maximum, respectively 7.6% and 10.3%), and in the control group, 8.34 ± 0.43% (minimum and maximum 7.6% and 9.2%), and there was no significant difference between the two groups before the procedure (P value: 0.59).

Density in the 3 months and 6 months follow-up after CXL in case group was 8.40 ± 0.60% (minimum and maximum, 7.4% and 9.5% respectively) and 8.35 ± 0.84% (minimum and maximum respectively 7.0% and 10.7%) and in the control group 8.27 ± 0.51% (minimum and maximum were 7.1% and 9.1%) and 8.33 ± 0.47% (minimum and maximum were 7.7% and 9.2%), which had no significant difference (P-value was 0.179 and 0.51, respectively). Hence, no significant difference in crystalline lens densitometry following CXL was seen 3 months and 6 months after surgery (P > 0.05). There was also no statistically significant difference in density of lens between the eyes underwent CXL and the fellow eyes of those patients in PNS (Table 1).

In the evaluating of Crystal Lens Densitometry using PNS system, all patients had a zero PNS in both eyes before surgery.

		Mean	Max - Min	P-Value
Before the procedure	CXL group	8.34 ± 0.67	10.3 - 7.6	0.59
	Control group	8.34 ± 0.43	9.2 - 7.6	
3 months after procedure	CXL group	8.40 ± 0.60	9.5 - 7.4	0.179
	Control group	8.27 ± 0.51	9.1 - 7.1	
6 months after procedure	CXL group	8.35 ± 0.84	10.7 - 7.0	0.51
	Control group	8.33 ± 0.47	9.2 - 7.7	

Table 1: *Densitometry of crystalline lens in eyes undergoing CXL and fellow eyes.*
(CXL: Corneal Cross Linking, Max: Maximum, Min: Minimum)

Discussion

This study showed that there is no statistically significant change in crystalline lens densitometry between the eyes which undergone CXL and naive eyes, before the procedure, 3 and 6 months follow up.

The complications of ultraviolet irradiation on the structure and function of the eye have been doubtful and newly have been investigated by epidemiological studies. In some studies, phototoxic effects of UV-A radiation on crystalline lens have been appraised. Various in vitro/in vivo studies also found out UV light as a risk factor for cataract formation and lens damage. Different hypotheses also explained the toxic effects through stress resulting from induced oxidative [19]. Azzam *et al*., (2003) was revealed that UV-A can cause senile cataracts formation and lens optics alteration through damage to lens cell membrane [18]. In addition, Hedge K.R. and coworkers (2007) showed UV as one of the main risk factors for radiation-induced cataract. They also indicated that these effects may be due to generation of ROS in crystalline lens and aqueous humour [20].

As already mentioned, CXL for developing post-LASIK ectasia and progressive KCN, has been proposed as a treatment modality [6-7, 28]. Similar to other novel therapeutic options, CXL also was found to be safe intervention. Previous studies have highlighted that a small amount of UV-A radiation in combination with riboflavin and in the presence of corneal thickness more than 400 μm, attain intra ocular tissues, including the lens and retina. These studies don't defend this point that UV-A causes damage to the intra ocular tissues during the CXL procedure [21].

Although CXL is commonly considered safe intervention but several long-term and short-term side effects of CXL have been reported and documented; these include bacteria, herpetic and acanthamoeba keratitis, delay in corneal epithelium improvement [8-10], corneal edema [11], corneal opacity [12], corneal thinning and toxicity [29-31], diminish corneal sensitivity [13-14], reduced permeability and drug penetration [32], limbal stem cell toxicity [15], sterile stromal infiltration [33], steady corneal edema due to endothelial cells loss [34].

Although a small amount of ultraviolet-B (UV-B) irradiation that can pass through the cornea is absorbed by high levels of ascorbic acid in aqueous humor, but a defined ratio can influence the crystalline lens epithelium, where it can be causative agent to damage. In addition, compared with UV-B, it is known that UV-A light can reach the nucleus, where it is absorbed by different chromophores to produce potentially damaging amount of reactive oxygen species (ROS) [35]. Moreover, ROS, which is formed in the cornea during operation, can be in contact with the lens when entering the acqous humor. Somehow that theoretically intraocular tissues can influenced by CXL procedure. However, possible complications on intra ocular tissues like retina and crystaline lens have not been fully evaluated. In a novel study transient functional and anatomical complication on macular profile were observed following CXL Which could confirm the intraocular penetration of the UV-A radiation in this intervention [16].

More recent *in vitro* studies have also shown that, exposure to UV light can be a possible risk factor to damaging the corneal endothelial tissues [35]. Additionally, several *in vivo* reports have raised concerns about endothelial cell damage induced by standard CXL surgery [11, 37]. Therefore, other intraocular complications may occur after CXL. This is also worth mentioning that toxic effects of CXL on the crystalline lens has not been fully understood and there are limited data available in the literature regarding the effects of CXL on the crystalline lens. To the best of our knowledge and based on the extensive review of the literature, current study one of the few researches investigating CXL complications on crystalline lens among patients with progressive KCN. We evaluated the crystalline lens density using Scheimpflug imaging and pentacam. Many studies with high numbers of patients have reported the repeatability and sensitivity of Scheimpflug camera technology and pentacam [37,38].

Our study have revealed that there is no statistically significant short term changes between the eyes underwent the CXL and the fellow eye. There is also no statistically significant changes in the eyes underwent CXL before the procedure and in the 3 and 6-months follow-up evaluations based on the Pentacam Scheimpflug imaging system. Our results are consistent with previous studies performed by Baradaran-Rafii., *et al.* [24], Vinciguerra., *et al.* [22] and Grewal., *et al* [23].

Baradaran-Rafii et al In a study on patients with progressive KCN who underwent standard CXL and 36 patients with non-progressive KCN as control group have showed that there is no difference between the two groups compared to baseline in lens densitometry [24].

In the study of Vinciguerra., *et al.* 24 eyes of 24 patients underwent the CXL procedure and Scheimpflug results with 36 months follow up have evaluated and data have determined that opacifications deterioration of the crystalline lens has not been changed [27]. In addition, in another study performed by Grewal., *et al.* on 102 patients with progressive KCN have found no changes in crystalline lens density 12 months after CXL intervention [28]. There were several limitations in the present study. First, our study have small sample size and it will be might difficult to find significant relationships from the data. Second, we just evaluated the short-term follow up for CXL complications and long-term side effects must be assessed.

Conclusion

In conclusion, results of current study showed that CXL did not have short-term cataractogenic complications in patients with progressive KCN. However, long-term complications remain uncertain and further study is needed to elucidate the CXL long-term complications. We recommend that future studies be designed with a larger number of participants and long-term follow up based on the physiopathology of UV-A induced oxidative stress and ROS measurements in anterior chamber and crystalline lens in human eyes after CXL.

Conflict of Interest

None to declare.

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