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Effect of Corneal Collagen Cross-Linkage on Intra-Ocular Pressure Measurement in Patients with Keratoconus

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Abstract

Keratoconus (KC) is progressively acknowledged as a complex, multifactorial, and heterogeneous condition that may stem from various independent metabolic and biochemical influences.

Corneal collagen cross-linking (CXL) with riboflavin (vitamin B2) combined with ultraviolet A (UVA) exposure has recently emerged as a novel treatment alternative. The objective of this study was to assess alterations in intraocular pressure (IOP) following CXL in a group of Iraqi individuals with keratoconus. This prospective investigation involved 28 patients with bilateral keratoconus (56 eyes) who received CXL using riboflavin and UVA light in both eyes. IOP assessments were conducted utilizing a Tonopen, and optical coherence tomography (OCT) was employed to assess corneal thickness right before treatment and at 3 and 6 months after treatment.

Intraocular pressure (IOP) showed a marked rise ($P<0.001$) following 3 and 6 months in comparison to baseline. The increase in IOP stayed significant ($P<0.001$) even after adjusting IOP readings for the reduction in corneal thickness (from 475.1 µm to 447.5 µm after 3 months and from 475.1 µm to 470.1 µm after 6 months). The increases in IOP percentage after three months were 11.2% before IOP correction and 27.1% after. The percentage rises in IOP after six months were 16.8% and 20.1% prior to and following IOP correction, respectively.

In summary, CXL using ribo-flavin and UVA light in keratoconus patients led to a notable rise in IOP as assessed by the Tonopen, even after accounting for variations in corneal thickness. This result could have occurred because of heightened corneal stiffness instead of an actual rise..

Keywords: Keratoconus, corneal collagen cross-linkage, intraocular pressure.

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Introduction

Keratoconus is a bilateral, non-inflammatory condition characterized by progressive thinning and bulging of the cornea. The cornea's conicalization results in irregular astigmatism, myopia, and diminished visual acuity (1). The degree to which heredity plays a role remains uncertain. The majority of patients lack a positive family history, and merely 10% of cases seem to impact offspring (2).

The exact cause of keratoconus is still unknown. However, it seems to be a heterogeneous condition that could stem from multiple unrelated metabolic and biochemical abnormalities (3). Due to its association with various genetic conditions (like Down's syndrome and Leber's congenital amaurosis), its occurrence among first-degree relatives, and its existence in monozygotic twins, keratoconus has been considered to possess a genetic factor for a long time (4). Just 10% of people with keratoconus have a familial background of autosomal dominant or recessive inheritance, the most common mode of transmission (3). In many instances, topographic imaging reveals that at least one eye is impacted (2).

Morphological signs of keratoconus consist of the formation of Fleischer's ring, a pigmented ring resulting from the aggregation of ferritin particles in the epithelium and expanded intercellular gaps (5); fissures in Bowman's membrane filled with collagen, cells, and Periodic Acid-Schiff-positive substances (6); thinning of the stroma along with abnormal keratocyte shapes (5); and variation in endothelial cell morphology (7). Keratoconus has been managed with a range of medical and surgical methods. These techniques, encompassing eyeglasses and soft contact lenses for initial conditions, and rigid gas permeable lenses (RGPs) for more severe instances, merely improve visual sharpness and do not slow down the pace of cone progression (5).

Keratoplasty, whether penetrating or deep anterior lamellar keratoplasty, serves as an alternative method for more severe cases, particularly those featuring considerable corneal scarring. Intracorneal ring segments (INTACS) can be utilized as they offer at least moderate enhancement in vision and increase tolerance for contact lenses (5).

Riboflavin (vitamin B2) combined with ultraviolet A (UVA) exposure is utilized in corneal collagen cross-linking (CXL), a treatment proven to decelerate, stabilize, or even reverse the advancement of corneal ectasia in individuals with keratoconus (8). The ability of collagen fibrils to create robust connections with adjacent fibrils is referred to as cross-linking. Collagen cross-linking happens naturally in the cornea as we grow older because of an oxidative deamination process in the end chains of collagen (9).

When used on the cornea, riboflavin functions as a photosensitizer stimulated by ultraviolet A light. The cornea grows stiffer due to the creation of robust chemical bonds between collagen fibrils caused by the light-triggered production of oxygen radicals. Reports indicate that the overall stiffness of human corneas may increase by as much as 330% (10). The most frequent complications of CXL encompass temporary stromal edema, corneal scarring, both transient and permanent haze, sterile infiltrates, and infectious keratitis (11). The present study aimed to assess IOP alterations after corneal collagen crosslinking in Iraqi individuals with keratoconus.

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Materials &Methods

Study Design

A forward-looking study took place at the Iraqi Red Crescent Hospital from May 2023 until May 2024. The study included 36 patients; 28 finished the study period, while 8 did not complete the follow-up. Twenty-eight patients with bilateral keratoconus (56 eyes) received cross-linking through riboflavin and UVA exposure in both eyes.

Inclusion criteria

Progressive keratoconus documented through Sirius tomography and/or multiple astigmatic refractions, with the thinnest corneal thickness at 400 µm or greater and a maximum keratometry (K) measurement under 58 diopters (D). No past eye surgery or other irregularities. History of advancement (rise in the maximum local K value of 1D or greater within one year), indicated by Sirius topographic measurements.

Exclusion Criteria

Patients were disqualified if they had any eye or systemic issue that might compromise the safety or validity of the study findings, such as corneal thickness under 400 µm, a history of herpetic keratitis due to the potential for reactivation, current ocular infection, significant corneal opacification or scarring, advanced ocular surface disease, a past of inadequate epithelial wound healing, autoimmune disorders, or the use of drugs known to hinder corneal healing or affect study outcomes.

Preoperative Assessment

The preoperative evaluation encompassed an analysis of overall medical and surgical history along with a comprehensive eye examination, featuring a slit-lamp examination and assessment of best-corrected visual acuity (BCVA) assessed with a Snellen chart, corneal thickness evaluated via optical coherence tomography (OCT), and intraocular pressure (IOP) measured using Tonopen. IOP readings were taken prior to treatment and at 3 and 6 months following treatment. IOP was recorded in millimeters of mercury (mmHg).

Procedure

The first step of CXL is topical anesthesia with tetracaine hydrochloride, followed by the mechanical removal of the central 9.0-mm corneal epithelium. Subsequently, riboflavin 0.1% is administered topically every 3 minutes for 30 minutes, then 30 minutes of UVA exposure is provided with a solid-state UVA light source. During UVA irradiation, 0.1% riboflavin is given every 5 minutes. The energy supplied is 3 mW/cm, and the diameter of the irradiation field is 8 mm.

Baseline IOP measurements taken before surgery are compared to those taken after surgery, with IOP adjusted for corneal thickness reduction at every follow-up visit (3 and 6 months postoperatively).).

The method of IOP measurement

A drop of topical anesthetic is given, and the patient is asked to focus on a target to reduce eye movement. The Tonopen AVIA is calibrated at the factory and doesn't need daily calibration; however, daily verification is necessary. Verification takes place by placing a tip cover, pressing the button until "dn" shows up, and then keeping the device in an upright position. A

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"Pass" verifies correct operation, whereas a "Fail" signifies the device must not be utilized. While measuring, the tonometer is positioned at a right angle to the cornea with slight contact and hand support on the patient's cheek. Following 4 seconds, the IOP and the statistical confidence indicator appear if a minimum of 6 applanations is noted.

Ten measurements are conducted for each eye, and the mean is determined. The application procedure needs to be restarted if any error codes show up on the LCD following the final beep. A statistical confidence level of 95 signifies that the standard deviation of the valid measurements is 5% or lower than the shown value. A measurement is regarded as more trustworthy when its statistical confidence indicator is elevated. It is recommended to redo the measurement if the statistical confidence indicator is 80 or less.

The primary researcher conducted all the readings. A drop of topical anesthetic is applied, and the patient is asked to focus on a target to reduce eye movement. The Tonopen AVIA comes precalibrated from the factory and doesn't need daily calibration, but daily verification is crucial. Verification is carried out by placing a tip cover, pressing the button until "dn" shows up, and then keeping the device in an upright position. A "Pass" indicates correct operation, whereas a "Fail" suggests the device is not safe for use.

While measuring, the tonometer is positioned at a right angle to the cornea with slight contact and hand support resting on the patient's cheek. After 4 seconds, the IOP and the statistical confidence indicator appear if a minimum of 6 explanations are documented. For each eye, ten measurements are obtained, and the mean is computed. The application process needs to be restarted if any error codes show up on the LCD following the final beep. A 95 statistical confidence indicator suggests that the standard deviation of the accurate measurements is 5% or lower than the shown value. A measurement is deemed more trustworthy when its statistical confidence indicator is elevated. It is recommended to repeat the measurement if the statistical confidence indicator is 80 or less. The primary researcher conducted all assessments.

Correction of IOP Measurements According to Corneal Thickness Changes

IOP readings need correction for accuracy, since variations in corneal thickness post-CXL can influence IOP measurements. Post-CXL, alterations in corneal thickness were assessed, and IOP was modified appropriately. The Sirius topographical instrument was utilized for adjustment. It integrates Scheimpflug imaging with Placido disk technology to create accurate maps of corneal thickness and curvature. The corneal thickness at its thinnest point and at the center was measured to calibrate IOP readings, enhancing the precision of post-CXL IOP evaluation. This adjustment reduces the effect of corneal thinning or thickening on tonometry readings, offering a more dependable assessment of IOP variations. Adhering to the protocol (11).

Statistical Analysis

Data input and analysis were conducted using version 24 of the Statistical Package for the Social Sciences (SPSS). A paired t-test was employed to evaluate the significance of the mean difference between pre- and post-treatment (prior to and following correction). A p-value lower than 0.05 was deemed significant.

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Results:

Baseline Patients Characteristics

This study included a total of 28 patients (56 eyes) with ages ranging from 18 to 28 years. The average age of the patients was 24.57 ± 2.82 years (expressed as mean ± standard deviation (SD)). There were 13 female patients (26 eyes; 46.4%) and 15 male patients (30 eyes; 53.6%).

Impact of Corneal CXL on IOP following 3 months

Table 1 demonstrated a statistically significant rise in IOP (P<0.001) when contrasting baseline IOP with post-CXL readings at the 3-month follow-up. The alteration showed an 11.2% rise in IOP following three months. Concerning alterations in corneal thickness post-CXL, the average corneal thickness reduced from 475.1 µm to 447.5 µm after three months, reflecting a mean change of -27.6 µm, while the IOP was adjusted for this variation. The IOP continued to be significantly elevated (P<0.001) following correction. The impact of CXL on IOP is likewise illustrated in Figure1.

Table 1: The Effect of CXL on IOP three months postoperatively:

Preoperative IOP	Postoperative IOP	Percent of Change	P-value
10.36±1.26	11.52±1.13 (before correction)	+11.2%	**<0.001**
	13.17±1.13 (after correction)	**+27.1%**	**<0.001**

Intraocular pressure (IOP) is measured as the mean ± standard deviation (SD) in mmHg. P-value <0.05 is considered significant.

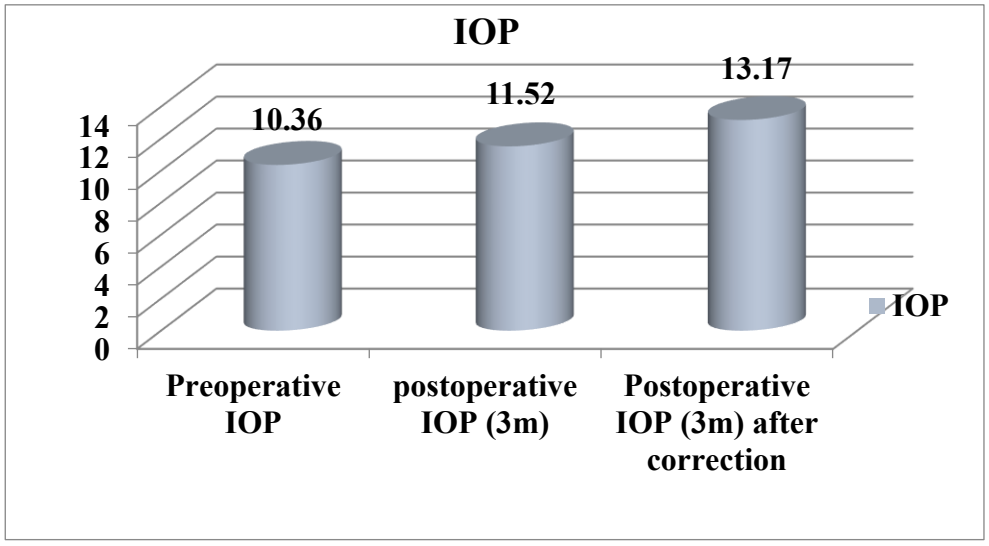

Figure 1: The Effect of CXL on IOP in patients with Keratoconus (after 3 months).

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Effect of Corneal Collagen Cross Linkage (CXL) on Intra-Ocular Pressure (IOP) after 6 months

Table 2 indicated a statistically significant rise in IOP (P<0.001) when comparing baseline IOP to post-CXL readings at the 6-month follow-up. The alteration was a (+16.8%) rise in IOP following six months.

In evaluating alterations in corneal thickness post-CXL, the average corneal thickness reduced from (475.1µm) to (470.1µm) after six months, resulting in a mean change of (-5.0µm). The IOP was adjusted for this alteration. Following the correction, the IOP continued to be significantly raised (P<0.001). The impact of CXL on IOP is also illustrated in Figure 2.

Table 2: The Effect of CXL on IOP six months postoperatively:

Preoperative IOP	Postoperative IOP	Percent of Change	P-value
10.36±1.26	12.1±0.86 (before correction)	+16.8%	**<0.001**
	12.44±0.86 (after correction)	**+20.1%**	**<0.001**

Intraocular pressure (IOP) is measured as mean ± (SD) and expressed in (mmHg).

P-value <0.05 is considered significant.

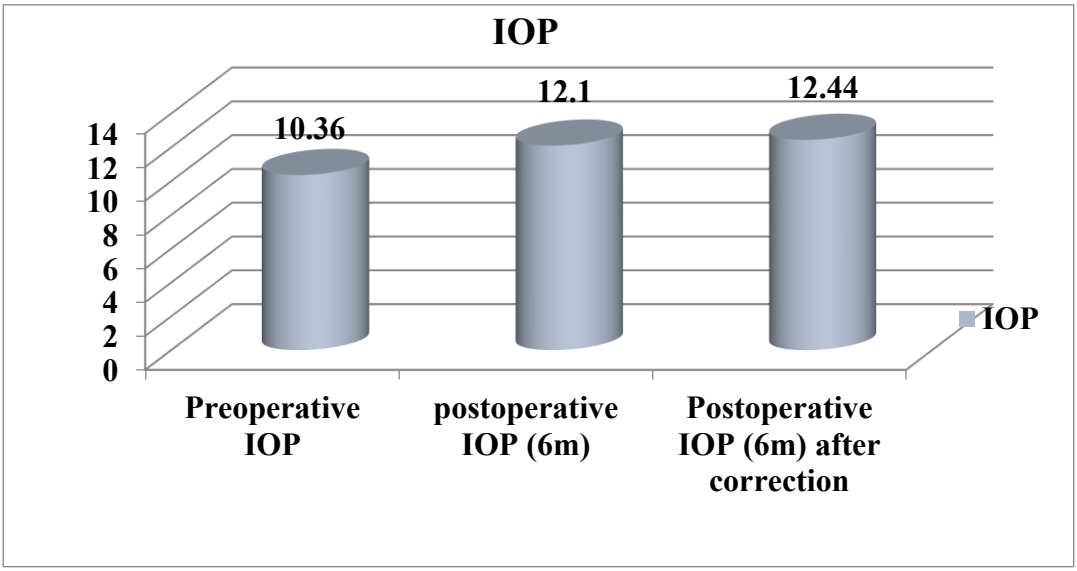

Figure 2: The Effect of CXL on IOP in patients with Keratoconus (after 6 months).

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Discussion

CXL involving riboflavin and UVA is a surgical technique employed to address keratoconus (12). The treatment depends on UVA triggering the photosensitizer riboflavin, which produces oxygen radicals that enhance chemical bonds among collagen fibrils, boosting corneal rigidity (13).

In this research, riboflavin and UVA CXL was conducted on 56 eyes diagnosed with keratoconus, and intraocular pressure was evaluated in each eye prior to CXL and at 3 and 6 months after CXL. After 3 months of CXL, there was a notable rise in IOP, reflecting a percentage change of (+11.2%) prior to IOP value adjustment and (+27.1%) following the adjustment.

Upon finishing the 6-month follow-up, we noted a sustained significant rise in IOP, with the percentage variation reaching approximately (+16.8%) prior to IOP value adjustment and (+20.1%) following the adjustment. It is well recognized that thin corneas lead to an underestimation of the measured IOP, while thick corneas result in an overestimation (14). An additional sign of enhanced corneal stability after CXL treatment is a rise in IOP (15). One explanation for the observed increase in pressure after CXL is that corneal stiffening is associated with enhanced corneal rigidity.

IOP readings may be influenced by the rigidity of the cornea, indicating the eye's flexibility. Increases in corneal rigidity and biomechanical alterations following CXL may result in elevated measured IOP in these individuals (16). Despite numerous studies indicating that changes in corneal elasticity and stiffness caused by CXL might overestimate IOP, a true increase in IOP due to CXL cannot be dismissed. A more invasive method for evaluating IOP could confirm this hypothesis (17).

This process might have a multifactorial cause, whether the increased IOP results from overestimation, a genuine rise in IOP, or a combination of both. Consequently, baseline IOP must be taken into account for patients receiving CXL.

The results of our study aligned with other research utilizing riboflavin and UVA CXL for keratoconus treatment and reporting corneal stiffness. Kymani's D et al. found that IOP measurements rose significantly at 6 and 12 months after treatment (both P<0.001). After CXL, changes in biomechanics and increased corneal rigidity are probably associated with alterations in IOP. No relationship existed between the patient's age and alterations in IOP at 6 and 12 months (18).

In the study by Livny E. et al., throughout all time points—one week, one month, and three months post-CXL therapy—IOP measurements were significantly elevated in the treated eye (0.005. This research discovered that the tonopen exaggerates IOP measurements post-CXL, likely because of the enhanced rigidity of the treated cornea (19).

The research conducted by Eissa I.M. et al. indicated that the assessed IOP rose significantly at 3, 6, and 12 months after CXL (P<0.001), likely resulting from enhanced corneal stiffness rather than an actual increase in IOP. Moreover, a direct correlation between preoperative central corneal thickness and postoperative IOP readings was found. However, there was no link between postoperative IOP measurements and patient gender or age (16).

The present research has multiple constraints. Pre-operative IOP values were not corrected for corneal thickness, which could lead to underestimation, while post-operative IOP was adjusted

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using Sirius topography data. The CXL procedure might have unintentionally influenced IOP readings, or the operation could have changed aqueous humor dynamics (decreasing outflow by impacting the trabecular meshwork). Additional elements, such as the use of corticosteroids and patient involvement, should be taken into account as well. Furthermore, this non-comparative research did not include a control group. Ultimately, the research depended on a sole tonometer (Tonopen), which might be affected by corneal biomechanics, constraining the generalizability of the IOP results.

Conclusion

In individuals with keratoconus, CXL using riboflavin and UVA exposure led to a notable rise in intraocular pressure (IOP) as assessed by the Tonopen, even after accounting for changes in corneal thickness. This observation was probably not a genuine rise but might be due to heightened corneal stiffness. Future studies ought to compare IOP readings taken with various tonometry instruments, including Goldmann applanation tonometry and the Ocular Response Analyzer (ORA), to confirm the results. These studies would assist in identifying if the IOP changes noted are specific to the device or indicative of genuine physiological alterations following CXL.

Ethical Approval

The study obtained ethical clearance from the Scientific Committee at Ibn Al-Haitham Teaching Hospital (no. 181, dated 25/4/2023). All participants provided verbal informed consent. Once we clarified the study's purpose and procedures to each participant, we secured their verbal consent prior to starting data collection.

Conflict of Interest: There are no significant financial or non-financial competing interests to disclose.

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Author contributions

All authors participated in sample gathering and the creation of the initial draft, and reviewed and endorsed the final version of the manuscript.

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