Research Article

Al-Iraqia Medical College Journal (AIMCJ)
ISSN (Online): 3104-4565
ISSN (Print): 3104-4557

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

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ARTICLE INFO
Received: 17/11 / 2025
Revised: 5/ 1/ 2026
Accepted: 6/ 1/ 2026
Publish online: 15 /4 / 2026

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CITATION
Ameer Maarouf Mansour, Ahmed Majeed Rasheed, Fadia Thamir Ahmed. Effect of Corneal Collagen Cross-Linkage on Intra-Ocular Pressure Measurement in Patients with Keratoconus. *AIMCJ*. 2026;3(1): 68-76.

DOI: https://doi.org/10.58564/AIMCJ3.1.2026.137

Abstract

Keratoconus (KC) is increasingly recognized as a complex, multifactorial, and heterogeneous disease that may arise from multiple independent metabolic and biochemical factors.

Corneal collagen cross-linking (CXL) using riboflavin (vitamin B2) with ultraviolet A (UVA) irradiation has recently been introduced as a new therapeutic option. This study aimed to evaluate intraocular pressure (IOP) changes after CXL in a sample of Iraqi patients with keratoconus. This prospective study included 28 patients with bilateral keratoconus (56 eyes) who underwent CXL with riboflavin and UVA irradiation in both eyes. IOP measurements were performed with a Tonopen, and optical coherence tomography (OCT) was used to evaluate corneal thickness immediately before treatment and at 3- and 6-month post-treatment.

Intraocular pressure (IOP) was significantly increased ($P<0.001$) after 3 and 6 months compared to baseline. The IOP rise remained significant ($P<0.001$) after correcting IOP measurements for corneal thickness decrease (from 475.1 µm to 447.5 µm after 3 months and from 475.1 µm to 470.1 µm after 6 months). The percentage increases in IOP after three months were 11.2% and 27.1% before and after IOP correction, respectively. The percentage increases in IOP after six months were 16.8% and 20.1% before and after IOP correction, respectively.

In conclusion, in patients with keratoconus, CXL with riboflavin and UVA irradiation resulted in a significant increase in IOP as measured with the Tonopen, even after correction for corneal thickness changes. This finding may have been due to increased corneal rigidity rather than a true increase.

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

AIMCJ. (2026),3; (1):68-76
https://doi.org/10.58564/AIMCJ3.1.2026.137

Introduction

Keratoconus is a non-inflammatory, bilateral disorder marked by progressive bulging and thinning of the cornea. Conicalization of the cornea leads to irregular astigmatism, myopia, and reduced visual acuity (1). The extent to which heredity contributes remains unclear. Most patients have no positive family history, and only 10% of cases appear to affect progeny (2).

The specific cause of keratoconus remains unknown. Nonetheless, it appears to be a heterogeneous disorder that may result from a variety of unrelated metabolic and biochemical defects (3). Given its correlation with other genetic disorders (such as Down's syndrome and Leber's congenital amaurosis), its incidence among first-degree relatives, and its presence in monozygotic twins, keratoconus has long been thought to have a genetic component (4). Only 10% of individuals with keratoconus have a family history of autosomal dominant or recessive transmission, which is the most prevalent mode of inheritance (3). In most cases, topographic imaging shows at least one eye affected (2).

Morphological indicators of keratoconus include the development of Fleischer's ring, a pigmented ring caused by ferritin particles accumulating in the epithelium and enlarged intercellular spaces (5); cracks in Bowman's membrane, packed with collagen, cells, and Periodic Acid-Schiff-positive material (6); thinning of the stroma and aberrant morphology of keratocytes (5); and endothelial polymorphism (7). Keratoconus has been treated using a variety of medical and surgical techniques. These methods, which include spectacles and soft contact lenses for early conditions and rigid gas permeable contact lenses (RGPs) for more advanced cases, only enhance visual acuity and do not reduce the rate of cone progression (5).

Keratoplasty, either penetrating or deep anterior lamellar keratoplasty, is another approach used for more advanced cases, especially those with significant corneal scarring. Intracorneal ring segments (INTACS) can also be used, as they provide at least moderate visual improvement and improve contact lens tolerance (5).

Riboflavin (vitamin B2) with ultraviolet A (UVA) irradiation is used in corneal collagen cross-linking (CXL), a therapeutic option shown to slow, stabilize, or even reverse the progression of corneal ectasia in patients with keratoconus (8). The capacity of collagen fibrils to form strong linkages with surrounding fibrils is called cross-linking. Collagen cross-linking naturally occurs in the cornea as we age due to an oxidative deamination process that occurs in collagen's end chains (9).

When applied topically to the cornea, riboflavin acts as a photosensitizer activated by ultraviolet A radiation. The cornea becomes more rigid as a result of the formation of strong chemical connections between collagen fibrils brought on by the light-induced generation of oxygen radicals. According to reports, the total stiffness of human corneas can grow by up to 330% (10). The most common complications of CXL include transient stromal edema, corneal scarring, transient and permanent haze, sterile infiltrates, and infectious keratitis (11). The current study was designed to evaluate IOP changes following corneal collagen crosslinking in Iraqi patients with keratoconus.

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Materials &Methods

Study Design

A prospective study was conducted at the Iraqi Red Crescent Hospital from May 2023 to May 2024. A total of 36 patients were included in the study; 28 completed the study period, and 8 did not complete follow-up. Twenty-eight patients with bilateral keratoconus (56 eyes) underwent CXL induced by riboflavin and UVA irradiation in both eyes.

Inclusion criteria

Documented progressive keratoconus by Sirius tomography and/or repeated astigmatic refraction, with corneal thickness at the thinnest point of 400 µm or more and a maximum keratometry (K) reading less than 58 diopters (D). No previous eye surgery or other abnormalities. History of progression (increase in the maximum local K value of 1D or more in 1 year), demonstrated by Sirius topography readings.

Exclusion Criteria

Patients were excluded if they had any ocular or systemic condition that could affect the safety or reliability of the study outcomes, including corneal thickness less than 400 µm, a history of herpetic keratitis due to the risk of reactivation, concurrent ocular infection, severe corneal opacification or scarring, severe ocular surface disease, a history of poor epithelial wound healing, autoimmune disease, or use of medications known to impair corneal healing or influence study results.

Preoperative Assessment

Preoperative assessment included a review of general medical and surgical history and a complete ophthalmologic examination, including slit-lamp examination, measurement of best-corrected visual acuity (BCVA) using a Snellen chart. chart, corneal thickness by optical coherence tomography (OCT), and intraocular pressure (IOP) by Tonopen. IOP measurements were performed before treatment and at 3 and 6 months after treatment. IOP was measured in millimeters of mercury (mmHg).

Procedure

Topical anesthesia with tetracaine hydrochloride is the initial step of CXL, followed by mechanical excision of the central 9.0-mm corneal epithelium. Afterward, topical riboflavin 0.1% is applied every 3 minutes for 30 minutes, followed by 30 minutes of UVA irradiation using a solid-state UVA illuminator. During UVA irradiation, 0.1% riboflavin is administered every 5 minutes. The energy delivered is 3 mW/cm, and the irradiation field has a diameter of 8 mm.

Preoperative baseline IOP measurements are compared with postoperative measurements, with IOP corrected for corneal thickness reduction at each visit (3 and 6 months postoperatively).

The method of IOP measurement

A topical anesthetic drop is applied, and the patient is instructed to fixate on a target to minimize eye movement. The Tonopen AVIA is factory-calibrated and does not require daily calibration, but daily verification is essential. Verification is performed by applying a tip cover, pressing the button until "dn" appears, then holding the device upright. A "Pass" confirms proper function, while a "Fail" indicates the

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device should not be used. During measurement, the tonometer is held perpendicular to the cornea with minimal contact and hand support on the patient's cheek. After 4 seconds, the IOP and the statistical confidence indicator are displayed if at least 6 applanations are recorded. Ten readings are taken per eye, and the average is calculated. The application process must be restarted if any error codes appear on the LCD after the last beep. When the statistical confidence indicator is 95, it indicates that the standard deviation of the valid measurements is 5% or less of the displayed value. A measurement is considered more reliable if its statistical confidence indicator is higher. It is advisable to repeat the measurement if the statistical confidence indicator is 80 or lower. The main researcher performed all readings. A topical anesthetic drop is applied, and the patient is instructed to fixate on a target to minimize eye movement. The Tonopen AVIA is factory-calibrated and does not require daily calibration, but daily verification is essential. Verification is performed by applying a tip cover, pressing the button until "dn" appears, then holding the device upright. A "Pass" confirms proper function, while a "Fail" indicates the device should not be used.

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Correction of IOP Measurements According to Corneal Thickness Changes

IOP measurements must be corrected to obtain accurate results, as changes in corneal thickness after CXL may affect IOP readings. After CXL, corneal thickness changes were measured, and IOP was adjusted accordingly. The Sirius topographical device was used to adjust. It combines Scheimpflug imaging with Placido disk technology to generate precise maps of corneal thickness and curvature. The measured corneal thickness at the thinnest point and the central cornea was used to adjust IOP readings, thereby improving the accuracy of post-CXL IOP assessment. This correction helps minimize the influence of corneal thinning or thickening on tonometry measurements, providing a more reliable evaluation of IOP changes. Following the procedure (11).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 24 was used for data input and analysis. A paired t-test was used to assess the significance of the difference in means between pre- and post-treatment (before and after correction). A p-value less than 0.05 was considered significant.

Results:

Baseline Patients Characteristics

A total of 28 patients (56 eyes) aged 18-28 years were included in this study. The mean age of the patients was 24.57 ± 2.82 years (presented as mean ± standard deviation (SD)). There were 13

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female patients (26 eyes; 46.4%) and 15 male patients (30 eyes; 53.6%).

Effect of Corneal CXL on IOP after 3 months

Table 1 showed a statistically significant increase in IOP (P<0.001) when comparing baseline IOP with post-CXL measurements at 3 months of follow-up. The change was an 11.2% increase in IOP after three months. Regarding corneal thickness changes after CXL, the mean corneal thickness decreased from 475.1 µm to 447.5 µm after 3 months, with a mean change of -27.6 µm, and the IOP was corrected for this change. The IOP remained significantly increased (P<0.001) after correction. The effect of CXL on IOP is also shown in Figure 1.

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.

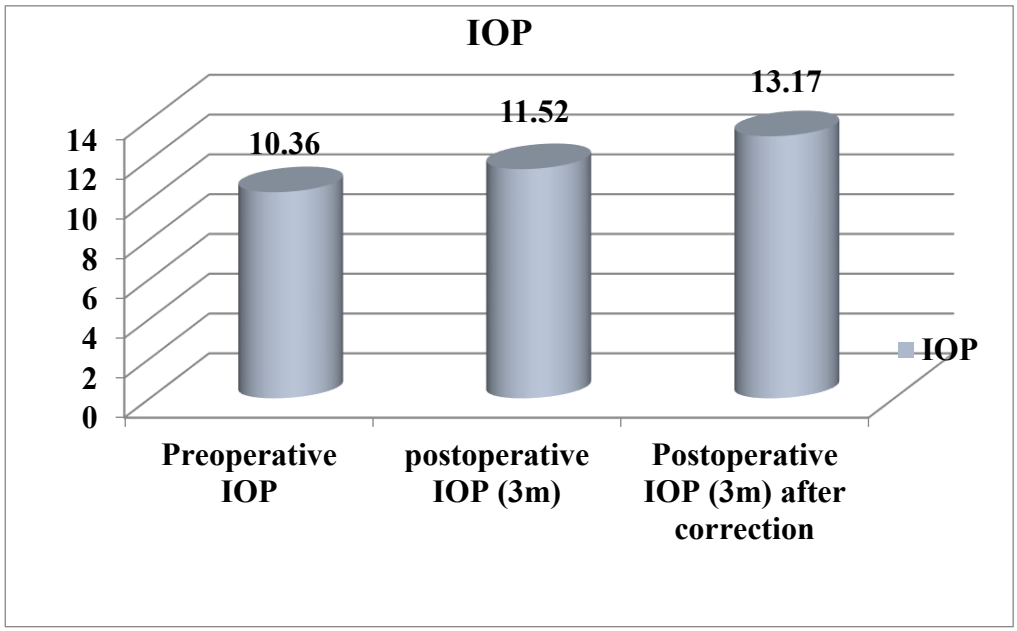

Figure 1: The Effect of CXL on IOP in patients with Keratoconus (after 3 months).

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https://doi.org/10.58564/AIMCJ3.1.2026.137

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

Table 2 showed a statistically significant increase in IOP (P<0.001) when comparing baseline IOP with post-CXL measurements at 6 months of follow-up. The change was a (+16.8%) increase in IOP after six months.

When considering changes in corneal thickness after CXL, the mean corneal thickness decreased from (475.1µm) to (470.1µm) after six months, with a mean change of (-5.0µm). The IOP was corrected for this change. After correction, the IOP remained significantly elevated (P<0.001). The effect of CXL on IOP is also shown 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.

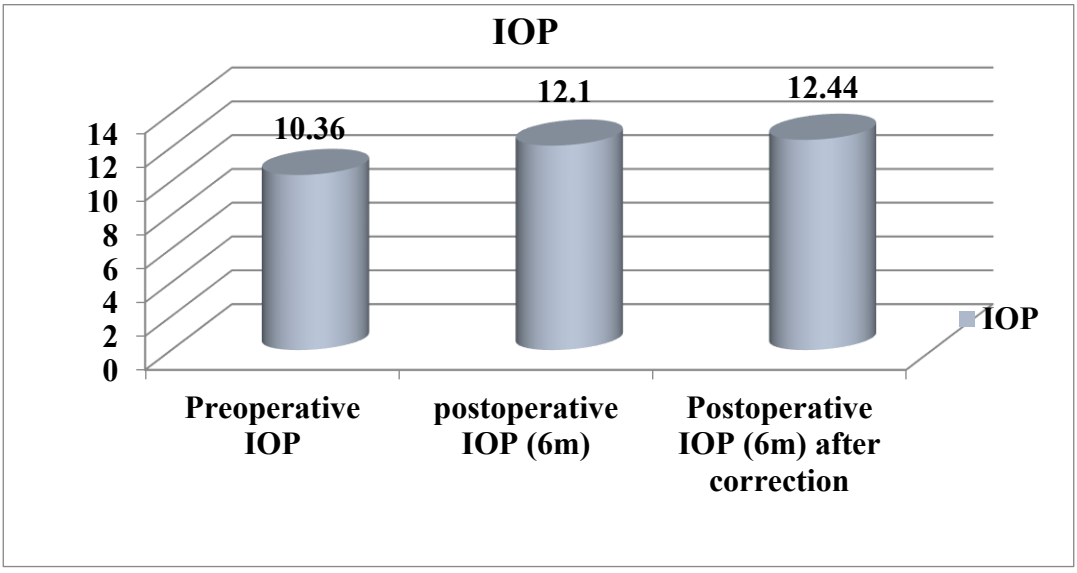

Figure 2: The Effect of CXL on IOP in patients with Keratoconus (after 6 months).

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Discussion

CXL with riboflavin and UVA is a surgical procedure used to treat keratoconus (12). The therapy relies on UVA activating the photosensitizer riboflavin, which generates oxygen radicals that strengthen chemical connections between collagen fibrils, increasing corneal stiffness (13).

In this study, CXL with riboflavin and UVA was performed in 56 eyes diagnosed with keratoconus, and IOP was assessed in each eye before CXL and at 3 and 6 months post-CXL. There was a significant increase in IOP after 3 months of CXL, with a percentage change of (+11.2%) before IOP value correction and (+27.1%) after correction.

After completing the 6-month follow-up, we observed a continued significant increase in IOP, with the percentage change reaching about (+16.8%) before IOP value correction and (+20.1%) after correction. It is widely acknowledged that thin corneas underestimate the observed IOP, whereas thick corneas exaggerate it (14). Another indication of improved corneal stability following CXL therapy is an increase in IOP (15). One theory explaining why measured pressure rises following CXL is that corneal stiffening is correlated with increased corneal rigidity.

IOP measurements can be affected by corneal stiffness, which reflects the eye's elasticity. Increases in corneal stiffness and biomechanical changes after CXL may lead to higher measured IOP in these patients (16). Although several studies suggest that CXL-induced changes in corneal elasticity and stiffness may overestimate IOP, a genuine rise in IOP with CXL cannot be ruled out. An invasive technique for assessing IOP might validate this hypothesis (17).

This process may have a multifactorial etiology, regardless of whether the elevated IOP is due to overestimation, a true rise in IOP, or both. Therefore, baseline IOP should be considered in patients undergoing CXL.

Our study's findings were consistent with other research using riboflavin and UVA CXL to treat keratoconus and report corneal stiffness. Kymani's D et al. reported that IOP readings increased significantly at 6 and 12 months following therapy (both $P<0.001$). Following CXL, biomechanical changes and increases in corneal stiffness are most likely linked to changes in IOP. There was no correlation between the patient's age and changes in IOP at 6 and 12 months (18).

In Livny E. et al.'s study, at all-time intervals, one week, one month, and three months after CXL therapy, IOP readings were substantially higher in the treated eye ($0.005<p<0.03$). This study found that the tonopen overestimates IOP readings after CXL, most likely due to the treated cornea's increased rigidity (19).

According to the study by Eissa I.M. et al., the measured IOP increased significantly at 3, 6, and 12 months following CXL ($P<0.001$), most likely due to an increase in corneal stiffness rather than a true rise in IOP. Additionally, a direct relationship between preoperative central corneal thickness and postoperative IOP measures was discovered. Nevertheless, there was no correlation between postoperative IOP readings and patient gender or age (16).

The current study has several limitations. Preoperative IOP values were not adjusted for corneal thickness, so they may be underestimated, although post-operative IOP was corrected using Sirius topography measurements. CXL treatment may have had an unforeseen effect on

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IOP measurements, or the surgery may have altered aqueous humor dynamics (reducing outflow by affecting the trabecular meshwork). Other factors, including corticosteroid use and patient participation, should also be considered. Additionally, this non-comparative study lacked a control group. Finally, the study relied on a single tonometer (Tonopen), which may be influenced by corneal biomechanics, limiting the generalizability of the IOP findings.

Conclusion

In patients with keratoconus, CXL with riboflavin and UVA irradiation resulted in a significant increase in intraocular pressure (IOP) as measured with the Tonopen, even after correcting for corneal thickness changes. This finding was likely not a true increase but may be attributable to increased corneal rigidity. Future research should compare IOP measurements obtained with different tonometry devices, such as Goldmann applanation tonometry or the Ocular Response Analyzer (ORA), to validate the findings. Such studies would help determine whether observed IOP changes are device-specific or represent true physiological changes after CXL.

Ethical Approval

The research received ethical approval from the Scientific Committee at Ibn Al-Haitham Teaching Hospital (no. 181, dated 25/4/2023). Verbal informed consent was obtained from all participants. After explaining the purpose and procedures of the study to each patient, we obtained their verbal consent before proceeding with data collection.

Conflict of Interest: There are no relevant financial or non-financial competing interests to report.

Funding: Nil

Author contributions

All authors contributed to sample collection and the preparation of the original draft, and read and approved the final version of the manuscript.

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