Three-year clinical observation of the outcomes of transepithelial and epithelial-off accelerated corneal collagen crosslinking treatment for different types of progressive keratoconus

JIN-RONG HUANG[1,2], HONG-FEI LIAO[1], CHUN-HONG WAN[2], LI-MIAO GONG[2],
LIANG-FEI HE[2], HONG-JI JIANG[2], BIAO LI[3] and YI SHAO[3]

[1]Department of Ophthalmology, The Affiliated Eye Hospital of Nanchang University, Nanchang, Jiangxi 330006;
[2]Department of Ophthalmology, The First Affiliated Hospital of Jiangxi Medical College, Shangrao, Jiangxi 334000;
[3]Department of Ophthalmology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330006, P.R. China

Abstract. In the present study, the clinical and long-term effects of accelerated transepithelial corneal collagen cross-linking (ATE-CXL) and accelerated epithelial-off corneal collagen crosslinking (A-CXL) for the treatment of different types of progressive keratoconus were compared. A total of 70 patients, including 96 eyes with advanced keratoconus, were enrolled in the study. ATE-CXL or A-CXL was performed on one or two eyes of each subject according to corneal thickness, keratoconus type and surgical approach. Patients were divided into the following four groups: Group A, ATE-CXL for central keratoconus; group B, A-CXL for central keratoconus; group C, ATE-CXL for peripheral keratoconus; and group D, A-CXL for peripheral keratoconus. Uncorrected distant visual acuity (UDVA), best-corrected distant (BD)VA and corneal astigmatism (CA) were evaluated in all patients by routine ophthalmology pre-operatively and 3 years post-operatively. Topographical features, including maximum corneal curvature (K_{max}), thinnest corneal thickness (TCT), anterior corneal elevation (ACE) and corneal endothelial cell density (ECD) were also compared across groups. The results suggested that pre- and post-operative UDVA, BDVA, K_{max}, CA and ACE values differed in all four groups (P<0.05), whereas no differences were observed between pre- and post-operative TCT and ECD (P>0.05). Concordant results were obtained between groups A and C and groups B and D. ATE-CXL achieved better control of central keratoconus UDVA, K_{max} and CA as compared with A-CXL. The difference between pre- and post-operative UDVA, K_{max} and CA as compared with A-CXL was highly correlated with the change in intraocular pressure and treatment effectiveness. There was a statistically significant improvement in BDVA with ATE-CXL for treatment of central keratoconus compared with that after A-CXL treatment (P=0.032). There were statistically significant improvements in BDVA (P=0.047), CA (P=0.045) and ACE (P=0.012) with A-CXL treatment of peripheral keratoconus when compared with ATE-CXL treatment. Central, and to a lesser extent, peripheral, keratoconus may be effectively controlled by either approach, with disease stabilization 3 years later. ATE-CXL is suggested to be the most suitable treatment for keratoconus of <400 μm with a corneal thickness of >400 μm; however, A-CXL yields superior long-term outcomes.

Introduction

Keratoconus, also known as corneal ectasia or corneal dilatation, is a non-inflammatory dilating disease of the eyes that affects the cornea; the clinical manifestations are progressive corneal thinning and protrusion leading to progressive myopia, irregular astigmatism and corneal scarring (1). Keratoconus is a disease in which the biomechanical properties of the cornea are gradually weakened by changes in corneal collagen fiber structure and extracellular matrix, as well as apoptosis of corneal cells (2). A recently developed technique known as riboflavin/ultraviolet (UV) corneal collagen cross-linking (CXL) may alleviate these pathological changes associated with keratoconus, thereby effectively preventing the development of secondary corneal dilation after keratoconus or refractive surgery (3); however, the standard early treatment plan is time-consuming. For patients with a thin cornea, there is also a risk of damaging corneal endothelial cells; thus, epithelial-off CXL should only be used in patients with a thick cornea.

Accelerated transepithelial CXL (ATE-CXL) is a novel and minimally invasive technique in which the use of high-irradiation pulsed UV light significantly reduces the treatment duration. In the present study, a pre- and post-control study design was used to evaluate changes in clinical characteristics after ATE-CXL, assess the safety and effectiveness of this

Correspondence to: Professor Hong-Fei Liao, Department of Ophthalmology, The Affiliated Eye Hospital of Nanchang University, 463 Bayi Avenue, Nanchang, Jiangxi 330006, P.R. China
E-mail: 13970098689@126.com

treatment and analyze the reproducibility and stability of the long-term effects on keratoconus progression.

Materials and methods

Subjects and grouping. Patients diagnosed with advanced keratoconus who underwent monocular or binocular ATE-CXL were enrolled between January 2014 and December 2017 in the present prospective, non-randomized, controlled clinical study. The inclusion criteria were as follows: i) Primary keratoconus, corneal topography indicating progressive enlargement of keratoconus lesions, maximal curvature increased by ≥1 D or optometry astigmatism increased by ≥1 D, or equivalent spherical degree increased by ≥0.5 D; ii) vision loss or corrected visual acuity (VA) <20/20; iii) at least one of the following positive signs on slit lamp examination: Thinning of the corneal stroma, tapered forward bulging, Fleischer ring, Vogt line, or epithelial or subepithelial scar; iv) central diopter of the corneal surface >47 D, difference between 3 mm below the corneal center and the upper 3 diopters >3 D, and difference between the central anterior surface of the cornea >1 D determined on corneal topographic examination. The exclusion criteria were as follows: Thinnest corneal thickness (TCT) <340 μm; endothelial cell density (ECD) <2,000/mm^2; post-elastic membrane rupture matrix edema; acute keratoconus; corneal trauma or secondary corneal ectasia after surgery; ocular diseases other than keratoconus and refractive error; systemic diseases affecting the eye, including collagen disease; and pregnancy. Ultimately, 70 cases with 96 eyes (48 males and 22 females; age range, 13-30 years) with the time from disease onset ranging from 0.52 to 120 months were included in the study. The follow-up time was 3 years. According to the Amsier-Krumeich grading standard and corneal thickness (4), different keratoconus types were divided into the following four groups according to the surgical method that was used: Group A, ATE-CXL central; group B, accelerated epithelial-off (A)-CXL central; group C, ATE-CXL peripheral; and group D, A-CXL peripheral. The treatment procedures were as follows: Group A, ATE-CXL for central keratoconus [maximum corneal curvature (K_{max})] <3 mm from the cornea; 340 μm <TCT<400 μm; n=22 cases, 34 eyes); group B, A-CXL for central keratoconus (K_{max} <3 mm from the cornea; TCT >400 μm; n=17 cases, 26 eyes); group C, ATE-CXL for peripheral keratoconus (K_{max} in the central 3 mm of the cornea, 340 μm <TCT <400 μm; n=16 cases, 18 eyes; and group D, A-CXL for peripheral keratoconus (K_{max} outside the 3 mm corneal area, TCT >400 μm; n=15 cases of 18 eyes).

Pre-operative examination. Follow-up examination included evaluation of uncorrected distant (UD)VA, best-corrected distant (BD)VA, apparent optometry, compensated intraocular pressure (IOPcc), K_{max}, mean corneal curvature (K_m), TCT, corneal astigmatism (CA), anterior corneal elevation (ACE) and corneal ECD.

TCT was measured by optical coherence tomography (Heidelberg Engineering Ltd.) using an Scheimpflug imaging corneal topographer (Pentacam HR software v.6.07r12; Oculus Optikgeräte GmbH). Corneal topography (Fig. 1) and corneal ECD were measured using a corneal endothelial cell counter (SP-2000P; Topcon Corporation).

ATE-CXL. The septum was opened and surface anesthetic was applied to the cornea, which was infiltrated with ParaCel solution (Avedro, Inc.) every 90 sec for a total induction time of 4 min. The corneal surface was rinsed and then infiltrated with VibeXXtra solution (0.25% riboflavin isotonic saline solution; Avedro, Inc.) every 90 sec for a total induction time of 6-10 min. After washing with balanced salt solution (BSS), the cornea was subjected to ATE-CXL with UV radiation cross-linking reinforcement. The irradiation intensity was 45 mW/cm^2, the irradiation spot diameter was 9 mm and a pulse irradiation mode was used (interval: 1 sec on/1 sec off). The total irradiation time was 320 sec and the total irradiation energy was 7.2 J/cm^2. During irradiation, 5% keratin solution (Shanghai Xianding Biotechnology Co., Ltd.) was applied to keep the corneal surface moist. The cornea was rinsed with BSS and eye drops were applied once. A sterile corneal bandage lens and an eye mask were applied to complete the procedure. There was no difference between the peripheral and central keratoconus operations and the eye position was not adjusted.

A-CXL. The septum was opened and after soaking the cornea with 20% alcohol for 15 sec under topical anesthesia, a 9-mm diameter corneal epithelial ring saw was used to remove the epithelium in the central region of the cornea. The cornea was covered with 0.1% riboflavin solution and the process was repeated every 90 sec for 10 min. The cornea was rinsed with BSS and A-CXL was performed by UVA radiation cross-linking reinforcement using an Avedro corneal remodeling platform (Avedro, Inc.) with the following settings: Spot diameter, 9 mm; pulse irradiation mode with pulse irradiation interval of 1 sec on/1 sec off; total energy, 7.2 J/cm^2; power intensity, 30 mW/cm^2; induction time, 10 min, UV pulse time, 8 min; and actual action time, 4 min. During irradiation, keratin solution was used to keep the corneal surface moist and afterwards, the cornea was rinsed with BSS. Eye drops were applied, followed by a sterile soft corneal bandage lens along with an eye mask. There was no difference between the peripheral and central keratoconus operations and the eye position was not adjusted.

Post-operative treatment and examination. At 1 day after surgery, antibiotics [levofloxacin (Clonidine) eye drops] were applied 4 times a day, 1 drop each time along with artificial tears (polyvinyl alcohol eye drops; Ruizhu PVA 20; Hubei Yuanda Tiantianming Pharmaceutical Co., Ltd.) once daily, 1 drop each time. When the epithelium had completely healed, the contact lens was removed and tobramycin dexamethasone eye drops were applied 4 times a day for 1 week. Timolol eye drops (Wuhan Wujing Pharmaceutical Co., Ltd) were applied twice per day and tobramycin dexamethasone eye drops (Hangzhou Minsheng Pharmaceutical Co., Ltd) were used once a week for 4 weeks until inflammation of the ocular surface subsided. During this period, patients were regularly examined and the cornea and IOP were closely monitored. UDVA, BDVA, apparent optometry, IOPcc, K_{max}, K_m, CA, TCT, ACE and ECD were recorded 3 years after the procedure.

Statistical analysis. Data were analyzed using SPSS v.20.0 software (IBM Corp.) and values are expressed as the mean ± standard deviation. Pre- and post-operative changes in
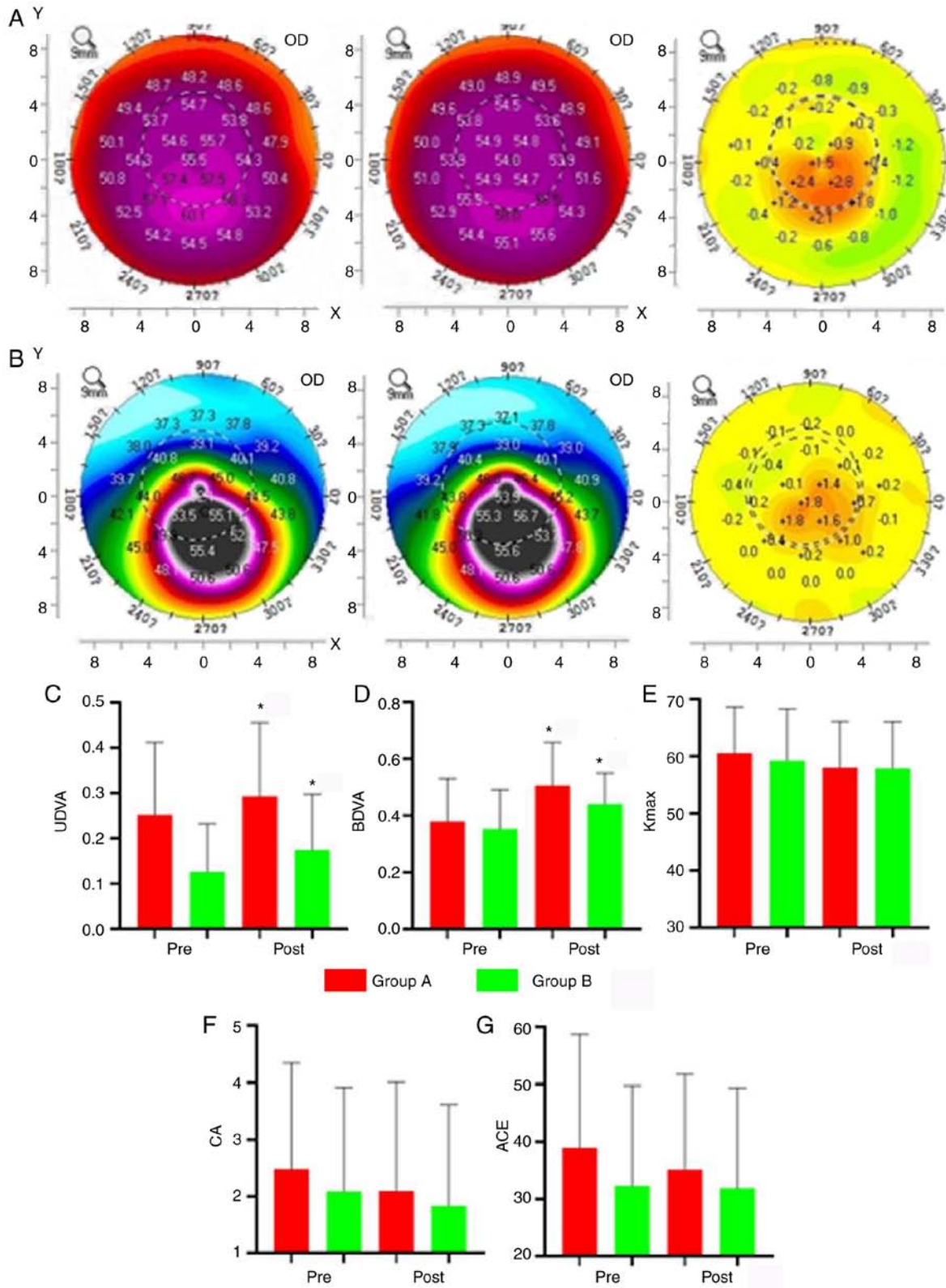
Figure 1. Pre- and post-operative results of groups A and B. (A) Left: Pre-operative topographic map of group A, indicating a steep lower central region with high curvature. Centre: After 3 years, the cornea had an eccentric oval shape with a steep area. Right: Differences between the pre- and post-operative topographic maps, which indicates that the high pre-operative curvature of the central region was decreased and flattened after surgery, whereas the low pre-operative curvature of the surrounding region was increased and became steeper. (B) Left: Pre-operative topographic map of group B, indicating high curvature in the lower region, lower curvature in the peripheral region and the lowest curvature in the central symmetric region. Centre: After 3 years, the cornea had an eccentric irregular shape with a steep region at the center and high curvature. Right: The differences between the pre- and post-operative topographic maps, which indicates that the curvature of the region with high pre-operative curvature was reduced and flattened, and that of the region with low pre-operative curvature was increased and became steeper. (C-G) Comparisons of parameters post-surgery with the baseline. (C) UDVA, and (D) BDVA were significantly decreased. (E) K_{max}, (F) CA and (G) ACE in groups A and B appeared decreased after the operation, though this was not significant. *P<0.05. Groups: A, accelerated transepithelial-CXL central; B, accelerated epithelial-off CXL central. CXL, corneal collagen crosslinking; Pre, prior to surgery; Post, following surgery; OD, optical density; UDVA, uncorrected distant visual acuity; ACE, anterior corneal elevation; BDVA, best-corrected distant visual acuity; CA, corneal astigmatism; ECD, corneal endothelial cell density; K_{max}, maximum corneal curvature.

Table I. Basic information of the patients at baseline.

Group	Patients (eyes)	Age (years)	Sex (male/female)	Onset (months)	K_{max} position (mm)	Corneal thickness (μm)
Total	70 (96)	20.5±4.1	48/22	20.5±25.5	2.6±1.7	430.68±56.33
A	22 (34)	19.6±3.2	12/10	20.4±27.8	2.1±0.8[b]	423.43±63.88[b]
B	17 (26)	21.5±2.4	16/1	21.4±26.8	1.8±0.7[a]	467.75±41.83[a]
C	16 (18)	18.5±2.3	13/3	20.5±22.6	4.6±1.3[a,b]	418.22±45.77[b]
D	15 (18)	19.5±5.1	7/8	22.5±23.4	4.3±1.5[a,b]	463.55±48.22[a]

[a]$P<0.05$, compared with group A, [b]$P<0.05$, compared with group B. Groups: A, ATE-CXL central; B, A-CXL central; C, ATE-CXL peripheral; D, A-CXL peripheral. ATE-CXL, accelerated transepithelial CXL; A-CXL, accelerated epithelial-off CXL; CXL, corneal collagen crosslinking.

the same group were analyzed with the paired-samples t-test, while one-way analysis of variance and Student-Newman-Keuls were was used for inter-group comparisons. The sex ratio was compared by χ^2 analysis, and t-test and binary logistic regression analysis were used to evaluate associations between UDVA, BDVA, K_{max}, CA, ACE, TCT and ECD. $P<0.05$ was considered to indicate statistical significance.

Results

Comparative analysis of general data between groups A and B and groups C and D. Regarding the clinical data, there were no statistically significant differences in terms of age, sex, onset, K_{max} position or corneal thickness between groups A and B and between groups C and D ($P>0.05$; Table I).

Post-operative outcomes of ATE-CXL and A-CXL for patients with central keratoconus. In group A, UDVA and BDVA differed significantly pre- vs. post-surgery ($P<0.05$; Table II); however, there were no significant changes in K_{max}, CA, TCT, ECD, IOP and ACE values ($P>0.05$). In group B, UDVA and BDVA differed significantly pre- vs. post-surgery surgery ($P<0.05$), but there were no changes in the values of K_{max}, CA, ACE, TCT or ECD ($P>0.05$; Table II). On the other hand, there was a significant correlation between the change in IOP and changes in BDVA and TCT after surgery as compared to the pre-operative values in group B. IOP was not significantly different between the post- and pre-operative stages in groups A and B (Fig. 2A and B).

Groups A and B did not differ in terms of change in UDVA value (post- vs. pre-operative; $P>0.05$); the changes in K_{max} in groups A and B were -5.4±8.0 and -4.4±7.0 D, respectively, and there was no significant difference between the two groups ($P>0.05$). Similarly, the two groups exhibited no difference in terms of the change in CA (-1.4±2.0 and -1.5±1.0 D, respectively; $P>0.05$). The change in ACE in groups A and B was -4.2±1.6 and -6.3±2.8 μm, respectively, and there was no statistically significant difference between the two groups either for ACE ($P>0.05$) or TCT ($P=0.20$) (Table II and Fig. 1).

Table II indicates that there was no statistical difference between group A and group B before treatment. After treatment, there was remission in both groups, but group A had a statistically significant improvement in BDVA ($P=0.032$) when compared with group B.

Post-operative outcomes of ATE-CXL and A-CXL in patients with peripheral keratoconus. The differences in UDVA, K_{max} and ACE prior to vs. after surgery in group C were statistically significant ($P<0.01$); however, there were no significant differences in BDVA, CA, TCT or ECD ($P>0.05$; Table II and Fig. 3). There was a significant correlation between changes in IOP and K_{max} after as compared to prior to treatment in group C (Fig. 2).

The differences between pre- and post-surgery UDVA, K_{max} and ACE in group D were significant ($P<0.05$); however, there was no significant difference in BDVA ($P>0.05$; Fig. 3 and Table II). Post-operative K_{max}, TCT and ECD values differed significantly from those prior to surgery ($P<0.05$; Fig. 3; Table II).

The change in UDVA (post- vs. pre-operative) was similar between groups C and D ($P>0.05$); however, changes in K_{max} differed significantly, with values of -2.5±1.8 and -3.2±2.8 D, respectively ($P=0.01$). The changes in CA values were also significant at -0.2±0.6 and -0.2±0.8 D, respectively ($P<0.05$). There were no differences in the changes in TCT, ACE and ECD between groups C and D ($P=0.20$; Table II).

Table III indicates that there was no statistical difference between group C and group D before treatment. After treatment, there was remission in both groups, but group D had a statistically significant improvement in BDVA ($P=0.047$), CA ($P=0.045$) and ACE ($P=0.012$) compared with group C.

The intergroup comparison shows that ATE-CXL achieved better control of central keratoconus UDVA, Kmax and CA as compared with A-CXL ($P<0.05$).

Discussion

The histopathologic changes observed in keratoconus are linked to the structural changes in corneal collagen fibers and biomechanical weakening caused by corneal cell apoptosis. The UV-riboflavin CXL procedure was developed by a research team at the University of Dresden, Germany for the treatment of keratoconus and has been indicated to increase the mechanical strength of collagen fiber and their capacity for resisting corneal expansion (4,5). In addition to increasing corneal hardness, cross-linking surgery may reduce resource utilization and thus alleviate the economic burden on patients (6). Since its first application to the treatment of progressive keratoconus in 2003, corneal collagen

Table II. Differences (post- vs. pre-operatively) in four groups.

A, Group A (n=34)

Parameter	Pre-operatively	Post-operatively	t	P-value
UDVA	0.25±0.16	0.28±0.17	-0.446	0.032
BDVA	0.38±0.18	0.50±0.19	-5.831	0.003
K_{max}	64.70±14.00	59.30±9.30	3.539	0.373
CA	4.80±3.10	3.60±2.00	2.700	0.061
TCT	423.43±63.88	410.35±59.87	2.511	0.304
ACE	38.80±20.30	33.70±14.50	1.693	0.063
ECD	2,376.38±132.71	2,224.15±174.22	2.888	0.054
IOP	15.13±1.50	16.13±1.40	2.228	0.079

B, Group B (n=26)

Parameter	Pre-operatively	Post-operatively	t	P-value
UDVA	0.12±0.09	0.15±0.11	-4.550	0.015
BDVA	0.38±0.12	0.44±0.16	-5.660	0.003
K_{max}	63.80±10.60	59.40±9.40	5.829	0.334
CA	4.50±3.00	3.50±2.30	2.189	0.069
TCT	467.75±41.83	452.75±38.48	1.190	0.270
ACE	35.40±16.20	33.80±15.40	0.248	0.232
ECD	2,637.48±141.57	2,589.52±153.32	1.095	0.723
IOP	14.98±1.90	16.03±1.70	1.838	0.072

C, Group C (n=18)

Parameter	Pre-operatively	Post-operatively	t	P-value
UDVA	0.22±0.14	0.25±0.14	-2.825	0.023
BDVA	0.28±0.19	0.41±0.18	-6.970	0.244
K_{max}	51.20±5.40	45.80±3.50	0.743	0.032
CA	2.30±1.80	2.10±1.50	6.050	0.570
TCT	418.22±45.77	402.33±43.42	1.056	0.837
ACE	36.60±14.70	30.80±12.30	2.749	0.035
ECD	2,508.26±252.63	2,343.16±110.05	6.088	0.099
IOP	16.07±0.42	16.49±0.54	0.772	0.445

D, Group D (n=18)

Parameter	Pre-operatively	Post-operatively	t	P-value
UDVA	0.16±0.12	0.24±0.15	-4.484	0.031
BDVA	0.41±0.20	0.57±0.15	-7.198	0.064
K_{max}	56.8±9.60	51.6±8.20	3.947	0.048
CA	2.40±2.00	2.00±1.90	2.281	0.032
TCT	463.55±48.22	442.36±38.44	11.039	0.110
ACE	34.6±13.80	32.5±17.20	0.288	0.042
ECD	2,496.45±162.38	2,324.43±165.67	2.154	0.131
IOP	14.34±1.36	15.71±0.54	2.493	0.177

Groups: A, accelerated transepithelial-CXL central; B, accelerated epithelial-off CXL central C, accelerated transepithelial CXL peripheral; D, accelerated epithelial-off CXL peripheral. ACE, anterior corneal elevation; BDVA, best-corrected distant visual acuity; CA, corneal astigmatism; CXL, corneal collagen crosslinking; ECD, corneal endothelial cell density; K_{max}, maximum corneal curvature; TCT, thinnest corneal thickness; UDVA, uncorrected distant visual acuity; IOP, intraocular pressure.
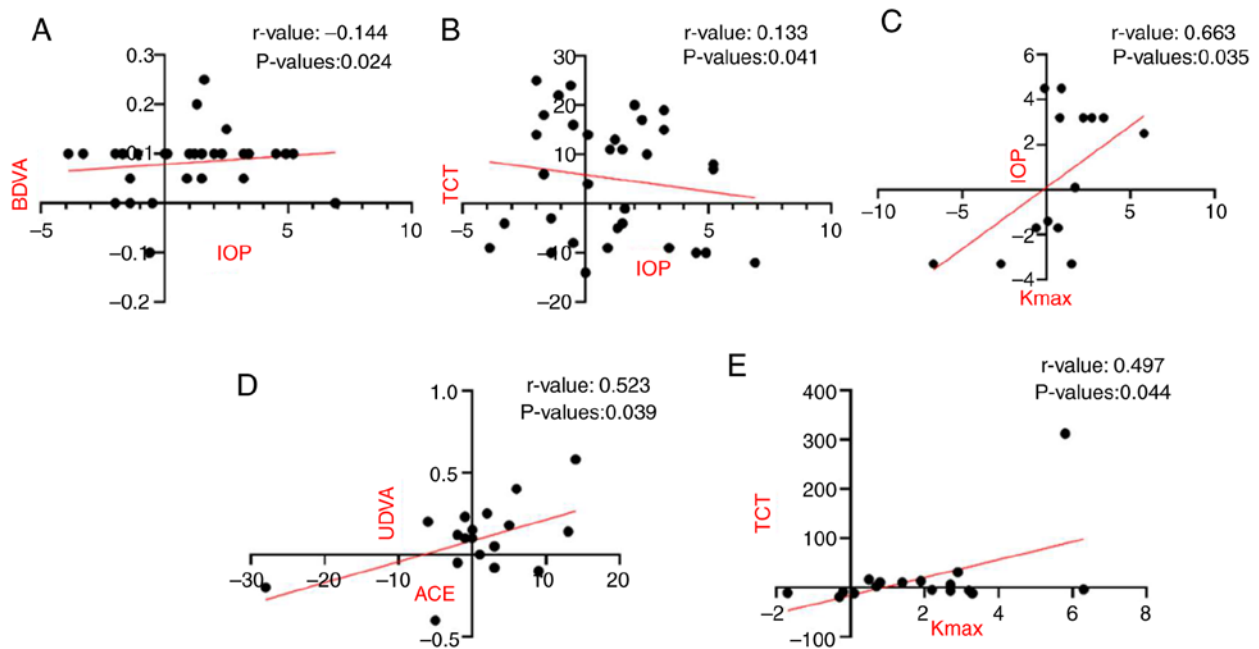
Figure 2. Correlation analysis. (A and B) In group A, the difference between pre- and post-operative ΔIOP values (A) decreased with increasing BDVA and (B) increased as a function of TCT. (C) In group C, the pre-operative K_{max} increased with greater changes in IOP. (D) In group D, the difference between pre- and post-operative ΔACE increased with the change in UDVA, while (E) the change in ΔK_{max} increased with greater change in TCT; these values were significantly correlated. Groups: A, ATE-CXL central; B, A-CXL central; C, ATE-CXL peripheral; D, A-CXL peripheral. ATE-CXL, accelerated transepithelial CXL; A-CXL, accelerated epithelial-off CXL; CXL, corneal collagen crosslinking; ACE, anterior corneal elevation; BDVA, best-corrected distant visual acuity; CA, corneal astigmatism; K_{max}, maximum corneal curvature; TCT, thinnest corneal thickness; UDVA, uncorrected distant visual acuity; IOP, intraocular pressure.

cross-linking has been expanded to include a variety of corneal diseases (7). The increased tensile strength of the corneal matrix after cross-linking increases corneal resistance to degradation by proteolytic enzymes, including matrix metalloproteinase and thermal damage (8-10). One study suggested that corneal topography and wavefront aberration values were stable at 7 years after cross-linking (11).

There are multiple methods of CXL. EDTA has been used in patients with keratoconus to enhance riboflavin penetration and TE-CXL; at the 12-month follow-up, safety indices, including average uncorrected VA, mean equivalent spherical reduction, mean simulated corneal curvature K-value and mean surface variance index were higher in the TE-CXL group compared with those in the control group (A-CXL) (12), indicating that TE-CXL effectively inhibits keratoconus progression.

The traditional CXL procedure includes removal of epithelial tissue with a central corneal diameter of 5-9 mm under topical anesthesia (13) to facilitate the entry of riboflavin into the corneal stroma. During the addition of riboflavin, the corneal surface temperature is constant and does not result in thermal burns to the corneal tissue (14). After irradiation, antibiotic eye ointment is applied and a contact lens soaked with 3 g/l ofloxacin is worn by the patient until the corneal epithelium has healed by 8% (15). The cross-linking therapy for keratoconus is relatively safe; the major post-operative complication is aseptic infiltration of the corneal stromal and scarring of the central cornea, which occur at estimated rates of 7.6 and 2.8%, respectively (16). For patients with a corneal thickness <400 μm, a hypotonic riboflavin preparation has been used to induce edema of the corneal stroma and thus

increase the thickness to >400 μm, after which cross-linking therapy achieved good results (17). An occasional complication is haze, which reduces the efficacy of steroid eye drops without affecting best-corrected VA (18). One patient developed diffuse lamellar keratitis after surgery and another developed herpes simplex keratitis that healed after treatment (19). Histological sections revealed an obvious boundary at a depth of 300 μm in the stromal layer, suggesting that the extent of cross-linking was limited to only the superficial tissue (17). Thus, the efficacy of cross-linking therapy for the posterior corneal cone remains to be determined; this is particularly important in the light of the fact that changes in corneal hardness and biomechanics after cross-linking therapy may increase the IOP (20).

Riboflavin/UV (370 nm) CXL is the first treatment that was indicated to effectively suppress keratoconus progression (3). The efficacy has been demonstrated experimentally and the technique lacks numerous disadvantages of other cross-linking methods (21). The adhesion of collagen fibers between adjacent layers of the keratoconus that are weakened by CXL was demonstrated by X-ray (22). In patients with a corneal thickness of >400 μm, CXL did not cause any damage to corneal endothelial cells and is therefore considered to be safe (7,23). It was also reported that CXL is able to increase corneal stress and strain, reduce swelling and increase the shrinkage temperature, as well as the tolerance of corneal stromal tissue to digestive enzymes (24-26). After CXL, the diameter of collagen fibers within 300 μm of the pre-corneal stroma was increased and no corneal endothelial cell damage occurred (27).

The present study indicated that the reduction in ACE persisted 3 years after ATE-CXL or A-CXL surgery,
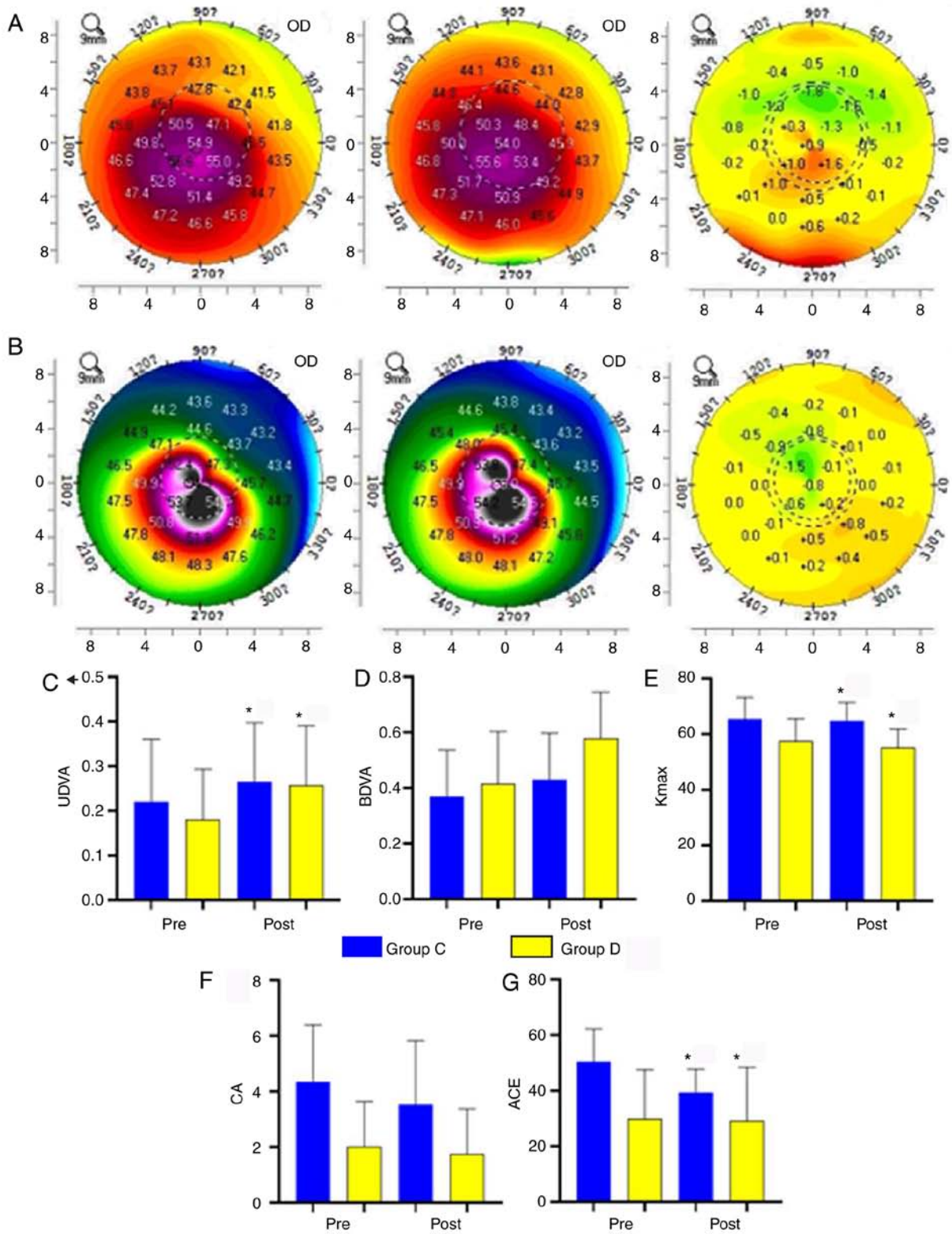
Figure 3. Pre- and post-operative results of groups C and D. (A) Left: Pre-operative topographic map of group C comprising a steep region on the temporal side with high curvature and low curvature in the peripheral region. Centre: After 3 years, the cornea had an eccentric irregular shape with a steep region in the center with high curvature. Right: Differences between the pre- and post-operative topographic maps, which indicates that the curvature of the topographic region with high pre-operative curvature continued to increase (i.e., became steeper), whereas the post-operative curvature was lower in areas with lower pre-operative curvature. (B) Left: Pre-operative topographic map of group D, revealing a steep region on the temporal side with high curvature and reduced curvature in the peripheral region. Centre: After 3 years, the cornea had an eccentric elliptical shape with a steep region in the center and high curvature. Right: Differences in the pre- and post-operative topographic maps, which indicates that the curvature of the region with high pre-operative curvature was reduced (i.e., became flattened), whereas that of the region with low pre-operative curvature was increased (i.e., became steeper). (C-G) Comparison of parameters post-surgery with the baseline. (C) UDVA was increased. (D) The increase in BDVA was insignificant. (E) K_{max} was decreased. (F) CA was not significantly changed. (G) ACE was significantly decreased. *P<0.05. Groups: C, accelerated transepithelial CXL peripheral; D, accelerated epithelial-off CXL peripheral. CXL, corneal collagen crosslinking; Pre, prior to surgery; Post, following surgery; OD, optical density; UDVA, uncorrected distant visual acuity; ACE, anterior corneal elevation; BDVA, best-corrected distant visual acuity; CA, corneal astigmatism; ECD, corneal endothelial cell density; K_{max}, maximum corneal curvature.

Table III. The differences (post-operative vs. pre-operative) between groups C and D.

Group C (n=18)

Examination Items	Pre-therapy	Post-therapy	t	P-value
UDVA	0.22±0.14	0.25±0.14	-2.825	0.023
BDVA	0.28±0.19	0.41±0.18	-6.97	0.244
Kmax	51.2±5.4	45.8±3.5	0.743	0.032
CA	2.3±1.8	2.1±1.5	6.05	0.570
TCT	418.22±45.77	402.33±43.42	1.056	0.837
ACE	36.6±14.7	30.8±12.3	2.749	0.035
ECD	2,508.26±252.63	2,343.16±110.05	6.088	0.099
IOP	16.07±0.42	16.49±0.54	0.772	0.445
Group D (n=18)				
UDVA	0.16±0.12	0.24±0.15	-4.484	0.031
BDVA	0.41±0.20	0.57±0.15	-7.198	0.064
Kmax	56.8±9.6	51.6±8.2	3.947	0.048
CA	2.4±2.0	2.0±1.9	2.281	0.032
TCT	463.55±48.22	442.36±38.44	11.039	0.110
ACE	34.6±13.8	32.5±17.2	0.288	0.042
ECD	2,496.45±162.38	2,324.43±165.67	2.154	0.131
IOP	14.34±1.36	15.71±0.54	2.493	0.177
P-value				
UDVA	0.109	0.884	-	-
BDVA	0.066	0.047	-	-
Kmax	0.068	0.066	-	-
CA	0.074	0.045	-	-
TCT	0.069	0.053	-	-
ACE	0.158	0.012	-	-
ECD	0.875	0.995	-	-
IOP	0.793	0.837	-	-

ACE, anterior corneal elevation; BDVA, best-corrected distant visual acuity; CA, corneal astigmatism; ECD, corneal endothelial cell density; K_{max}, maximum corneal curvature; TCT, thinnest corneal thickness; UDVA, Uncorrected distant visual acuity.

suggesting that the hardness of the corneal matrix fibers and tensile strength were increased, whereas the curvature was decreased by the cross-linking. At 3 years after CXL, most patients in the ATE-CXL group had improved UDVA and BDVA and reduced K_{max}, CA, and ACE; however, the differences in K_{max}, CA and ACE were attenuated compared with those in the A-CXL group, indicating that the transepithelial treatment was less effective than the epithelial approach.

After the surgery, UDVA and BDVA were significantly increased in groups A and B. A superior curative effect was observed in group A, which was reflected in the K_{max} value. On the other hand, group B exhibited greater improvements in CA and ACE, indicating that while ATE-CXL and A-CXL may be used to treat central keratoconus, the latter achieves superior results.

In groups C and D, UDVA was increased, whereas K_{max} and CA were decreased after CXL. VA of the naked eye was higher in group C than in group D but K_{max} and CA were lower.

This provides further evidence that the transepithelial method is the less effective of the two cross-linking methods, particularly in patients with peripheral keratoconus.

Intra-group correlation analysis revealed that the difference between pre- and post-operative IOP in group C decreased with increasing difference in BDVA, whereas that of BC increased with increasing difference in TCT. TCT, IOP and diopter are presumed to be associated, although this is debated. The corneal thickness is reduced in myopia (28) and it has been determined that for each 70-μm decrease, the IOP as measured with a tonometer is 3.5 mmHg lower than the baseline value (29); however, other studies have not indicated any correlation between diopter and IOP (30). By contrast, a larger diopter was suggested to be associated with higher IOP (31). In the present study, a correlation was observed between diopter and IOP (P<0.05) that may be linked to the decrease in corneal thickness caused by reduced IOP, which increases TCT and results in a superior curative effect. In group C, a larger change in IOP was correlated with a greater change in K_{max}. The

changes in intraocular pressure and BDVA in group B before and after operation were larger than those in group A, which indicated that treatment received by Group B patients was more effective. In group D, the differences between pre- and post-operative UDVA and ACE values were correlated, which may be attributed to alterations in corneal shape caused by post-operative changes in ACE; however, further experimental data are required to confirm this possibility.

As conical cornea is a progressive complication of corneal degeneration, it is unable to self-heal, and the incidence and progression rate of the two eyes in the same patient are different. The progression rate in different patients also varied. Therefore, a pre- and post-control study design was adopted in the present study. By acute observation, changes in patients with ATE-CXL (UVA parameters: 45 mW/cm^2 x320 sec, 7.2 J/cm^2) and A-CXL (UVA parameters: 30 mW/cm^2 x480 sec, 7.2 J/cm^2) were assessed, and observation and analysis of long-term effects and associated factors of ATE-CXL in advanced keratoconus treatment were performed to verify the long-term safety and effectiveness of the surgical treatment of progressive cone cornea. The predictiveness and stability of outcomes determined in the present study are expected to contribute to the guidance and provide a reference for the clinical treatment of pyramidal cornea.

Of note, the present study had certain limitations. The left and the right eyes of the patients were not distinguished at the time of sample collection, which may have affected the accuracy of the results. In future experiments, the left or right eye should be used consistently in order to improve the accuracy of the experimental results.

In conclusion, ATE-CXL and A-CXL are safe and effective treatment methods for inhibiting keratoconus progression after 3 years. Central (and to a lesser extent, peripheral) keratoconus may be effectively controlled by either approach, with disease stabilization 3 years later. ATE-CXL is associated with fewer adverse reactions and better patient compliance, which may reduce post-operative pain and complications caused by epithelialization. ATE-CXL is the most suitable treatment for keratoconus of <400 μm with a corneal thickness of >400 μm; however, A-CXL yields superior long-term outcomes. The relatively small sample size precluded observation of the outcomes of ATE-CXL in patients with secondary keratoconus after refractive surgery. Larger studies with longer follow-up are required to verify the effectiveness of ATE-CXL for controlling keratoconus.

Availability of data and materials

The datasets generated and/or analyzed in this study are available from the corresponding author on reasonable request.

Authors' contributions

JRH and YS designed the study. CHW, LMG, LFH, HFL, BL and HJJ collected and analyzed the data. JRH wrote the manuscript. All authors read and approved the final manuscript.

Ethical approval and informed consent

The study protocol was approved by The Medical Research Ethics Committee of the Affiliated Eye Hospital of Nanchang University (Nanchang, China). The experiments were performed according to relevant guidelines and regulations. All study subjects were informed of the study purpose and design and provided written informed consent prior to their participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Shalchi Z, Wang X and Nanavaty MA: Safety and efficacy of epithelium removal and transepithelial corneal crosslinking for keratoconus. Eye (Lond) 29: 15-29, 2015.
2. Baum J: On the location of the cone and the etiology of keratoconus. Cornea 14: 142-143, 1995.
3. Wollensak G: Crosslinking treatment of progressive keratoconus: New hope. Curr Opin Ophthalmol 17: 356-360, 2006.
4. Alió JL and Shabayek MH: Corneal higher order aberrations: A method to grade keratoconus. J Refract Surg 22: 539-545, 2006.
5. Prausnitz MR and Noonan JS: Permeability of cornea, sclera, and conjunctiva: A literature analysis for drug delivery to the eye. J Pharm Sci 87: 1479-1488, 1998.
6. Hoyer A, Raiskup-Wolf F, Spörl E and Pillunat LE: Collagen cross-linking with riboflavin and UVA light in keratoconus.results from Dresden. Ophthalmologe 106: 133-140, 2009 (In German).
7. Wollensak G, Spoerl E and Serler T: Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 135: 620-627, 2003.
8. Santhiago MR, Giacomin NT, Medeiros CS, Smadja D and Bechara SJ: Intense early flattening after corneal collagen cross-linking. J Refract Surg 31: 419-422, 2015.
9. Sel S, Nass N, Pötzsch S, Trau S, Simm A, Kalinski T, Duncker GI, Kruse FE, Auffarth GU and Brömme HJ: UVA irradiation of riboflavin genetates oxygen-dependent hydroxyl radicals. Redox Rep 19: 72-79, 2014.
10. Kopsachilis N, Tsaousis KT, Tsinopoulos IT, Kruse FE and Welge-Luessen U: A novel mechanism of UVA and ribollavin-mediated corneal cross-linking through induction of tissue transglutaminases. Cornea 32: 1034-1039, 2013.
11. O'Brart DP: Corneal collagen cross-1inking: A review. J Optom 7: 113-124, 2014.
12. Leccisotti A and Islam T: Transepithelial corneal collagen cross-linking in keratoconus. J Refract Surg 26: 942-948, 2010.
13. Caporossi A, Baiocchi S, Mazzotta C, Traversi C and Caporossi T: Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A rays induced cross-linking of corneal collagen: Preliminary refractive results in an Italian study. J Cataract Refract Surg 32: 837-845, 2006.

14. Mencucci R, Mazzotta C, Rossi F, Ponchietti C, Pini R, Baiocchi S, Caporossi A and Menchini U: Riboflavin and ultraviolet A Collagen crosslinking: In vivo thermographic analysis of the corneal surface. J Cataract Refract Surg 33: 1005-1008, 2007.
15. Spoerl E, Mrochen M, Sliney D, Trokel S and Seiler T: Safty of UVA-riboflavin cross-linking of the cornea. Cornea 26: 385-389, 2007.
16. Koller T, Mrochen M and Seiler T: Complication and failure rates after corneal crosslinking. J Cataract Refract Surg 35: 1358-1362, 2009.
17. Hafezi F, Mrochen M, Iseli HP and Seiler T: Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refract Surg 35: 621-624, 2009.
18. Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C and Caporossi A: Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: In vivo confocal microscopic evaluation. Clin Exp Ophthalmol 35: 580-582, 2007.
19. Kymionis GD, Portaliou DM, Bouzoukis DI, Suh LH, Pallikaris AI, Markomanolakis M and Yoo SH: Herpetic keratitis with iritis after corneal crosslinking with riboflavin and ultraviolet A for keratoconus. J Cataract Refract Surg 33: 1982-1984, 2007.
20. Nagy ZZ: Laser in situ keratomileusis combined with topography-supported customized ablation after repeated penetrating keratoplasty. J Cataract Refract Surg 29: 792-794, 2003.
21. Spöerl E, Huhle M, Kasper M and Seiler T: Increased rigidity of the cornea caused by intrastromal cross-linking. Ophthalmologe 94: 902-906, 1997 (In German).
22. Meek KM, Tuft SJ, Huang Y, Gill PS, Hayes S, Newton RH and Bron AJ: Changes in collagen orientantion and distribution in keratoconus corneas. Invest Ophthalmol Vis Sci 46: 1948-1956, 2005.
23. Wollensak G, Spoerl E, Wilsch M and Seiler T: Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit. J Cataract Refract Surg 29: 1786-1790, 2003.
24. Spoerl E, Wollensak G and Seiler T: Increased resistance of crosslinked cornea against enzymatic digestion. Curr Eye Res 29: 35-40, 2004.
25. Spoerl E, Wollensak G, Dittert DD and Seiler T: Thermomechanical behaviour of collagen-cross-linked porcine cornea. Ophthalmologica 218: 136-140, 2004.
26. Wollensak G, Spoerl E and Seiler T: Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg 29: 1780-1785, 2003.
27. Wen D, Song B, Li Q, Tu R, Huang Y, Wang Q, McAlinden C, O'Brart D and Huang J: Comparison of epithelium-Off versus transepithelial corneal collagen cross-linking for keratoconus: A systematic review and meta-analysis. Cornea 37: 1018-1024, 2018.
28. Kim EJ, Sajjad A, Montes de Oca I, Koch DD, Wang L, Weikert MP and Al-Mohtaseb ZN: Refractive outcomes after multifocal intraocular lens exchange. J Cataract Refract Surg 43: 761-766, 2017.
29. Whitacre MM, Stein RA and Hassanein K: The effect of corneal thickness on applanationn tonometry. Am J Ophthalmol 115: 592-596, 1993.
30. Bonomi L, Mecca E and Massa F: Intracular pressure in myopic anisometropia. Int Ophthalmol 5: 145-148, 1982.
31. Wong TY, Klein BE, Klein R, Knudtson M and Lee KE: Refractive errors, intraocular pressure, and glaucoma in a white population. Ophthalmology 110: 211-217, 2003.