Long-term Outcomes of Transepithelial Accelerated Corneal Collagen Crosslinking in Patients with Progressive Keratoconus

Resultados a Longo Prazo de *Crosslinking* Transepitelial Acelerado em Doentes com Queratocone Progressivo

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ABSTRACT

INTRODUCTION: Our purpose was to systematically evaluate the long-term effectiveness of transepithelial accelerated corneal collagen crosslinking (TE-ACXL) in the treatment of eyes with progressive keratoconus (KC) throughout a 4-year follow-up.

METHODS: Eyes of patients who underwent TE-ACXL (6 mW/cm^2 for 15 minutes) for progressive KC and presented 48 months of follow-up were included. Corrected distance visual acuity (CDVA), keratometry measurements (Kmax, maximum keratometry, Kmean, mean keratometry and Astg, corneal astigmatism), thinnest corneal thickness (PachyMin), and topographic, and tomographic indexes (specifically the PRC, posterior radius of curvature from the 3.0 mm centered on the thinnest point of the cornea, and the D-index) were analysed preoperatively and every 12 months after TE-ACXL, up to 48 months.

RESULTS: Forty one eyes from 30 patients were included, with a mean age at crosslinking of 20.90±4.69 years. Eleven eyes were excluded from the comparison between 48 months and baseline values because they were submitted to either an epithelial-off crosslinking (n=6), an intracorneal ring segment (n=4) or a corneal penetrating transplant (n=1), after 36 months of follow-up. There was a significant increase in Kmean (+0.64±1.04 D, p<0.001; +0.98 ± 1.49 D, p<0.001; +1.27±2.01 D, p<0.001; +1.13±2.00 D, p=0.006) and D-index (+0.51±1.03 units, p=0.007; +0.69±1.25 units, p=0.002; +1.02±1.72 units, p=0.002; +1.15±1.64 units, p<0.001) throughout follow-up. PRC decreased significantly throughout follow-up (-0.12±0.22, p=0.002; -0.15±0.24, p<0.001; -0.17±0.43, p=0.021; -0.16±0.43, p=0.027). PachyMin decreased significantly at 36 and 48 months (-8.50±15.93 µm, p=0.004; -7.82±18.37, p=0.033). CDVA improved significantly at 12 and 48 months (-0.10±0.29 logMAR units, p=0.045; -0.17±0.33 logMAR units, p=0.013), but not at 24 or 36 months. Surgery and follow-up were uneventful in all subjects, regarding potential complications of this procedure.

CONCLUSION: TE-ACXL is a safe treatment for progressive KC. However, there may still be a significant variation in topographic, tomographic, and pachymetric parameters in these eyes in a 4-year period following the procedure. Therefore, further procedures may be required to halt disease progression.

KEYWORDS: Corneal Cross-Linking; Corneal Topography; Cross-Linking Reagents; Disease Progression; Keratoconus.

RESUMO

INTRODUÇÃO: Avaliação sistematizada da efetividade a longo prazo do *crosslinking* transepitelial acelerado (TE-ACXL) no tratamento de olhos com queratocone (KC) progressivo, ao longo de um período de 4 anos.

MÉTODOS: Foram incluídos olhos com KC progressivo submetidos a TE-ACXL (6 mW/cm^2 durante 15 minutos), com seguimento de 48 meses. Foram avaliados os seguintes parâmetros: melhor acuidade visual para longe corrigida (CDVA), parâmetros queratométricos (queratometria máxima (Kmax), queratometria media (Kmean) e astigmatismo corneano (Astg)), espessura corneana mínima (PachyMin), índices topográficos e tomográficos (nomeadamente o raio de curvatura posterior dos 3 mm centrados no ponto mais fino da córnea (PRC) e o índice D). Estes parâmetros foram analisados pré-operatoriamente e a cada 12 meses após o TE-ACXL, até 48 meses após a cirurgia.

RESULTADOS: Foram incluídos 41 olhos de 30 doentes. A idade média pré-operatória foi 20,90±4,69 anos. Foram excluídos 11 olhos da comparação entre os resultados aos 48 meses e os resultados pré-TE-ACXL, visto que estes olhos foram submetidos a outros procedimentos 36 meses após a cirurgia inicial (nomeadamente, *crosslinking* com remoção do epitélio (n=6), segmentos de anel intracorneanos (n=4) ou queratoplastia penetrante (n=1). Registou-se um aumento significativo da Kmean (+0,64±1,04 D, p<0,001; +0,98 ± 1,49 D, p<0,001; +1,27±2,01 D, p<0,001; +1,13±2,00 D, p=0,006) e do índice D (+0,51±1,03 unidades, p=0,007; +0,69±1,25 unidades, p=0,002; +1,02±1,72 unidades, p=0,002; +1,15±1,64 unidades, p<0,001) ao longo do seguimento. O PRC diminuiu significativamente ao longo dos 48 meses (-0,12±0,22 mm, p=0,002; -0,15±0,24 mm, p<0,001; -0,17±0,43 mm, p=0,021; -0,16±0,43 mm, p=0,027). A PachyMin diminuiu significativamente aos 36 e aos 48 meses (-8,50±15,93 µm, p=0,004; -7,82±18,37, p=0,033). A CDVA melhorou significativamente aos 12 e aos 48 meses (-0,10±0,29 unidades logMAR, p=0,045; -0,17±0,33 unidades logMAR, p=0,013), mas não aos 24 meses ou aos 36 meses. A cirurgia e o seguimento dos doentes decorreram sem intercorrências em todos os doentes incluídos.

CONCLUSÃO: O TE-ACXL é um tratamento seguro para o KC progressivo. Contudo, pode ocorrer uma variação significativa dos parâmetros topográficos, tomográficos e paquimétricos nestes olhos, num período de 4 anos após o procedimento. Assim, podem ser necessários outros procedimentos para abrandar a progressão da doença.

PALAVRAS-CHAVE: Crosslinking Corneano; Progressão da Doença; Queratocone; Reagentes de Crosslinking; Topografia da Córnea.

INTRODUCTION

Keratoconus (KC) is a progressive, bilateral, and asymmetric ectatic disease of the cornea, usually presenting in the second to third decades of life.[1,2] Central or paracentral corneal stromal thinning occurs, accompanied by apical protrusion (ectasia), with consequent mild to significant visual impairment.[2,3] It is the most frequent form of corneal ectasia, with an estimated incidence of up to 1 in 20004 in the general population, with more recent studies suggesting a prevalence that can be as high as 4.79% in general pediatric patients.[5,6] Currently, corneal tomography and biomechanics are the most useful tools in its early diagnosis,[7] while topographic, tomographic, and pachymetric parameters are usually combined to monitor disease progression.[8,9] The "Global Consensus on Keratoconus and Ectatic Diseases" defined ectasia progression as a consistent change in ≥2 of the following parameters: (1) steepening of the anterior corneal surface; (2) steepening of the posterior corneal surface; (3) thinning and/or an increase in the rate of corneal thickness change from the periphery to the thinnest point. Furthermore, the consensus panel agreed that a change in both uncorrected distance visual acuity (UDVA) and corrected distance visual acuity (CDVA) is not required to document progression, and that while specific quantitative data are lacking to further define progression, these data would likely be machine/technology specific.[10] Some parameters are frequently used as KC progression criteria

in the literature, in studies that use the Pentacam® tomography system (OCULUS Optikgeräte GmbH, Wetzlar, Germany).[8,11-16] However, these parameters have not been validated. Furthermore, using one isolated parameter is not in agreement with the global consensus on KC.[10]

Keratoconus treatment has evolved greatly in recent years.[17] Corneal collagen crosslinking (CXL) has emerged as an effective treatment in decreasing or even aborting disease progression. It is a safe and minimally invasive procedure that increases corneal biomechanical rigidity and is the only form of treatment that specifically targets the disease pathophysiology.[18,19] The first established CXL protocol – the Dresden Protocol – was described by Wollensack *et al*[20] and is considered the standard (or conventional, C-CXL) procedure. It involves epithelial debridement to facilitate stromal riboflavin absorption, application of a 0.10% riboflavin 5-phosphate solution for 30 minutes, followed by exposure to UVA radiation (365 nm, 3 mW/cm^2) for 30 minutes, enabling a total fluence of 5.4 J/cm^2.[20] Nonetheless, corneal epithelium debridement and prolonged corneal exposure are responsible for the main adverse effects and disadvantages of C-CXL (infection, sub-epithelial haze, sterile corneal infiltrates, corneal scarring, endothelial damage, postoperative pain, and delayed visual rehabilitation).[19,21-23] To reduce these negative outcomes, new accelerated protocols emerged (A-CXL). These are based on the Bunsen-Roscoe law of photochemical reciprocity, therefore maintaining the cumulative dose of radiation administered. Posteriorly, to avoid the possible complications associated with epithelial debridement, transepithelial protocols (TE-CXL) were developed.[19,23-25] Notwithstanding, transepithelial accelerated crosslinking (TE-ACXL) efficacy may be limited by: (1) the epithelial barrier to riboflavin diffusion and UVA and oxygen absorption by the corneal stroma; (2) the assumption that increasing irradiation intensity during a reduced period has the same biological effect in the cornea.[26,27] Furthermore, some studies report an inferior efficacy of the TE-ACXL regarding visual and topographic outcomes, when compared with the standard C-CXL,[28,29] while others report similar outcomes with both techniques and even defend the transepithelial technique due to its better visual outcomes and lower postoperative complications.[30] Therefore, long-term efficacy and safety TE-ACXL studies are required to fully determine the efficacy of this technique in progressive KC and which patients will benefit from it. This study aims to answer the following research question: is TE-ACXL effective in halting the progression of progressive keratoconus over a four-year period.

METHODS

STUDY DESIGN

This article was redacted according to the recommendations of The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement.

The authors conducted a retrospective observational cohort study of eyes with progressive keratoconus who underwent TE-ACXL (6 mW/cm^2 for 15 minutes), from January 2016 to July 2019, and were followed for 48 months afterward in the Ophthalmology Department of Centro Hospitalar Universitário de São João (Porto, Portugal). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Centro Hospitalar Universitário de São João. Written informed consent was obtained from all patients or legal guardians (in patients under the age of consent) before surgical interventions. This study's inclusion criteria were defined as: age between 14 and 32, pachymetry at its thinnest point (PachyMin) ≥ 380 µm and previously documented progression of keratoconus. The exclusion criteria were apical corneal scarring, severe dry eye, delayed epithelial healing, active ocular infections, connective tissue disease, pregnancy or lactation, and previous history of cornea surgery.

All clinical, visual, corneal topographic, tomographic, and pachymetric parameters from the eyes included in the study were evaluated preoperatively and every 12 months postoperatively up to 48 months. Our primary outcome was the variation of CDVA, Kmax, Kmean, PRC, PachyMin, and D-index. CDVA was recorded via a Snellen chart and converted to logarithm of minimal angle of resolution (logMAR) units to allow statistical analysis. Kmax, Kmean, Astg, PRC, PachyMin, index of height decentration (IHD), index of vertical asymmetry (IVA), index of surface variance (ISV), keratoconus index (KI), and Belin/Ambrósio D-index were determined using the Pentacam HR® (OCULUS Optikgeräte GmbH, Wetzlar, Germany). The Keratoconus Classification was determined in accordance with the Pentacam HR® Ktc Scoring System. The baseline score for all parameters was defined as the preoperative measurement closest to the date of the procedure; the presence of postoperative KC progression represented treatment failure. Disease progression was assessed at 12, 24, 36, and 48 months after TE-ACXL.

SURGICAL TECHNIQUE AND POSTOPERATIVE CARE

All operations were conducted under sterile conditions in an operative room. Oxybuprocaine hydrochloride 4 mg/L eyedrops were used for preoperative local anesthesia. TE-ACXL was carried out through an intact epithelium; to do so, a TE riboflavin preparation (0.25% riboflavin, ethylenediamine tetra-acetic acid [EDTA], trometamol [Tris], benzalkonium chloride [BAC]) and a 0.45% phosphate buffer saline preparation were instilled in the cornea every 3 minutes for 30 minutes preoperatively. UV-A irradiation began after corneal stromal saturation was confirmed through slit-lamp assessment of the anterior chamber flare. The cornea was exposed to a UV-A light beam at an intensity of 6 mW/cm^2 for 15 minutes to achieve a total dose intensity of 5.4 J/cm^2. During this period, a riboflavin solution and a sterile balance sodium solution were administered alternatively every 3 minutes to avoid corneal dehydration. After the procedure, antibiotic eye drops (ofloxacin 0.30%) were prescribed for a week, as well as topical steroids eye drops (fluorometholone 0.10%) for 2 weeks and sodium hyaluronate 0.20% as needed. Regarding the follow-up, visits were scheduled for day 1 postoperatively, at 3 months, and every 6 months afterward.

STATISTICAL ANALYSIS

The sample's characteristics were summarized, and data were exposed as counts and proportions for categorical variables. Continuous variables were described as mean and standard deviation (or median and interquartile range, when distributions were skewed). The postoperative variation in visual, keratometric, pachymetric, topographic, and tomographic parameters was calculated by subtracting their baseline values from the subsequent readings at each follow-up visit (therefore, positive delta values denote an increase in that parameter, whilst negative delta values represent a decrease). Paired t-tests were used to compare preoperative and postoperative outcomes; multiple related samples were compared via within-subjects ANOVA test. A *p*-value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS Statistics software (version 29.0 for Mac OS; SPSS Inc., Chicago, IL, USA).

ETHICS

The study was approved by the Institutional Ethics Review Board of Centro Hospitalar Universitário de São João, Porto, Portugal. The protocol conformed with the canons of the Declaration of Helsinki for research involving human participants, as well as the European Union's General Data Protection Regulation. Informed consent was waived in view of the retrospective nature of the study.

RESULTS

Forty one eyes from 30 patients were included in this study. Our cohort included 22 male and 8 female patients; 11 patients received TE-ACXL in both eyes (9 male and 2 female patients). The mean age was 20.90 ± 4.69 years (ranging from 14 to 32). Further baseline characteristics are shown in Table 1. Regarding KC, its preoperative mean was 2.66 ± 0.66 and its preoperative distribution is displayed in Fig. 1. The most frequent keratoconus grade was 3 (N=18, 43.9%), the second being 2, 2.5, 3.5 (all with an n=6, 14.6%). All 41 eyes completed the 48-month follow-up. However, 11 eyes were excluded from the comparison between 48 months and baseline values because they were submitted to either an epithelial-off crosslinking (n=6), an intracorneal ring segment (n=4), or a corneal penetrating transplant (n=1), after 36 months of follow-up. Variations (Δ) between baseline visual, keratometric, pachymetric, topographic, and tomographic corneal parameters at 12, 24, 36, and 48 months postoperatively are presented in Table 2. All surgical procedures were carried out uneventfully and no complications were registered throughout follow-up.

Table 1. Baseline demographic, clinical, visual, corneal topographic, tomographic and pachymetric characteristics of patients undergoing transepithelial accelerated crosslinking.

Parameter	n = 30 patients
Patients [male:female]	30 [22:8]
Age at crosslinking	20.90 ± 4.69
Eye rubber	9 (30)
Allergic conjunctivitis	10 (33.3)
Atopy	11 (36.7)
Asthma	2 (6.7)
Parameter	**n = 41 eyes**
CDVA (logMAR)	0.47 ± 0.36
K1 (D)	46.98 ± 3.72
K2 (D)	50.51 ± 4.45
Astg (D)	3.53 ± 1.91
Kmax (D)	57.83 ± 5.71
Kmean (D)	48.66 ± 3.97
PachyMin (µm)	453.20 ± 35.97
ISV	101.35 ± 30.83
IVA	1.09 ± 0.39
IHD	0.16 ± 0.06
KI	1.27 ± 0.12
D-Index	9.99 ± 3.21
KC	2.66 ± 0.66

Categorical data are presented as *n* (%). Continuous data are presented as mean ± standard deviation (SD), or median ± interquartile range for skewed data.
µm, micrometer; Astg, astigmatism (K2-K1); CDVA, corrected distance visual acuity; D, diopter; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; K1, flat keratometry; K2, steep keratometry; KI, keratoconus index; KC, keratoconus classification; Kmax, maximum keratometry; Kmean, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry.

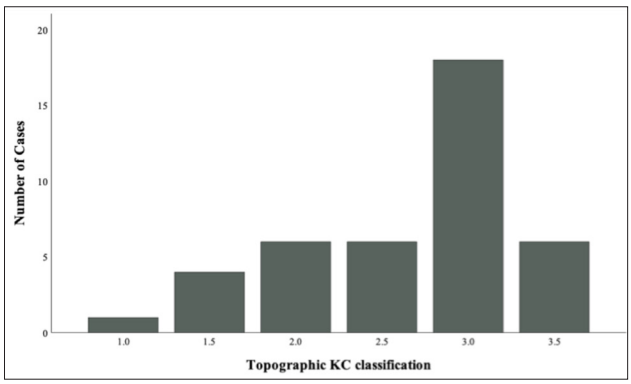

Figure 1. Distribution of baseline keratoconus classification.

VISUAL ACUITY

Fig. 2 shows CDVA variation over time (in logMAR values). The mean CDVA preoperatively was 0.47 ± 0.36 logMAR units. Mean variation was -0.10 ± 0.20 logMAR units at 12 months (p=0.045), -0.10 ± 0.33 logMAR units at 24 months (p=0.099), -0.07 ± 0.34 logMAR units at 36 months (p=0.226), and -0.17 ± 0.33 logMAR units at 48 months postoperatively (p=0.013).

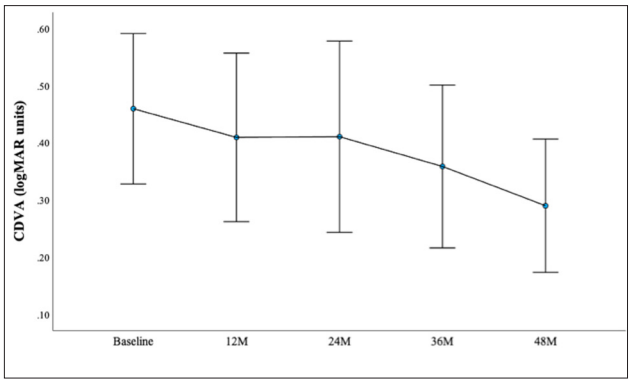

Figure 2. Corrected distance visual acuity (CDVA) in LogMAR units at baseline, 12, 24, 36, and 48 months after trans-epithelial accelerated crosslinking (TE-ACX).

TOPOGRAPHY AND TOPOGRAPHIC INDICES

The baseline mean Kmax was 57.83 ± 5.71 D. No significant changes were found during follow-up, except at 36 months. The mean variation was +0.45 ± 2.07 D (p=0.211) at 12 months, +0.58 ± 2.16 D (p=0.119) at 24 months, +1.08 ± 2.40 D (p=0.013) at 36 months, and +0.61±3.03 D (p=0.299) at 48 months postoperatively. Baseline average Kmean was 48.66 ± 3.97 D. There was a significant increase in Kmean throughout follow-up. Its mean variation was +0.64 ± 1.04 D at 12 months (p<0.001), +0.98 ± 1.49 D at 24 months (p<0.001), +1.27 ± 2.01 D at 36 months (p<0.001), and +1.13 ± 2.00 D at 48 months (p=0.006). As for Astg, no statistically significant differences were found throughout follow-up (Table 2). There were no significant variations in ISV, IVA, IHD, or KI throughout follow-up.

TOMOGRAPHY, TOMOGRAPHIC INDICES, AND PACHYMETRY

The preoperative mean PachyMin value was 453.20 ± 35.97 µm. There were no statistically significant variations in the first 24 months of follow-up (-1.69 ± 12.96 µm at 12 months, p=0.447; -15.25 ± 69.03 µm at 24 months, p=0.194). There was a significant increase in the D-index throughout follow-up. The baseline value was 9.99 ± 3.21 units. At 12 months, there was a significant increase of +0.51 ± 1.03 (p=0.007). At 48 months there was a significant increase of +0.69 ± 1.25 units (0.002). At 36 months there was a significant increase of 1.02 ± 1.72 (p=0.002). Finally, at 48 months, there was a significant increase of 1.15 ± 1.64 (p<0.001). A significant decrease of PachyMin was determined at 36 months follow-up (-8.50 ± 15.93 µm; p=0.004), and at 48 months follow-up (-7.82 ± 18.37; p=0.033). Regarding PRC, there was a significant decrease in this parameter throughout follow-up (-0.12 ± 0.22 at 12 months; p=0.002; -0.15 ± 0.24 at 24 months; p<0.001; -0.17 ± 0.43 at 36 months; p=0.021; -0.16 ± 0.43 at 48 months; p=0.027).

Table 2. Mean changes in visual, corneal tomographic, topographic and pachymetric parameters between 12, 24, 36, and 48 months and baseline values.

Variables	Post-TE-ACXL			
	Δ 12-months		Δ 24-months	
	N = 35		N = 36	
	Mean ± SD	p value	Mean ± SD	p value
CDVA (logMAR)	-0.10 ± 0.29	0.045	-0.10 ± 0.33	0.099
Astg (D)	0.02 ± 1.05	0.924	0.03 ± 1.09	0.855
Kmax (D)	0.45 ± 2.07	0.211	0.58 ± 2.16	0.119
Kmean (D)	0.64 ± 1.04	<0.001	0.98 ± 1.49	<0.001
PRC (mm)	-0.12 ± 0.22	0.002	-0.15 ± 0.24	<0.001
PachyMin (µm)	-1.69 ± 12.96	0.447	-4.25 ± 15.31	0.105
ISV	0.15 ± 11.58	0.941	0.37 ± 11.73	0.852
IVA	-0.02 ± 0.16	0.385	-0.05 ± 0.17	0.079
IHD	-0.003 ± 0.03	0.526	-0.002 ± 0.02	0.531
KI	0.004 ± 0.05	0.687	-0.001 ± 0.04	0.830
D-index	0.51 ± 1.03	0.007	0.69 ± 1.25	0.002
Variables	**Post-Operation**			
	Δ 36-months		Δ 48-months	
	N = 34		N = 28	
	Mean ± SD	p value	Mean ± SD	p value
CDVA (logMAR)	-0.07 ± 0.34	0.226	-0.17 ± 0.33	0.013
Astg (D)	0.08 ± 1.33	0.721	0.02 ± 1.46	0.949
Kmax (D)	1.08 ± 2.40	0.013	0.61 ± 3.03	0.299
Kmean (D)	1.27 ± 2.01	<0.001	1.13 ± 2.00	0.006
PRC (mm)	-0.17 ± 0.43	0.021	-0.16 ± 0.43	0.027
PachyMin (µm)	-8.50 ± 15.93	0.004	-7.82 ± 18.37	0.033
ISV	2.70 ± 15.45	0.323	-0.21 ± 18.42	0.951
IVA	-0.03 ± 0.20	0.396	-0.06 ± 0.25	0.207
IHD	-0.005 ± 0.03	0.391	-0.012 ± 0.05	0.200
KI	0.01 ± 0.07	0.342	-0.01 ± 0.06	0.677
D-index	1.02 ± 1.72	0.002	1.15 ± 1.64	<0.001

Note: 11 eyes were excluded from the comparison between 48 months and baseline values because they were submitted to either an epithelial-off crosslinking (n=6), an intracorneal ring segment (n=4) or a corneal penetrating transplant (n=1), after 36 months of follow-up.

µm, micrometer; Astg, astigmatism (K2-K1) CDVA, corrected distance visual acuity; D, diopter; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; KI, keratoconus index; Kmax, maximum keratometry; Kmean, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; PRC, posterior radius of curvature from the 3.0 mm centered on the thinnest point of the cornea; TE-ACXL, transepithelial accelerated corneal collagen crosslinking; SD, standard deviation.

DISCUSSION

In our retrospective study, 41 eyes from 30 patients underwent TE-ACXL (with chemical enhancers for stromal riboflavin diffusion, and an UV-A light beam intensity of 6 mW/h for 15 minutes to achieve a total dose intensity of 5.4 J/cm^2) due to progressive KC. These patients were followed for 48 months. Eleven eyes required further procedures, ≥36 months after the initial TE-ACXL, undergoing either an epithelial-off crosslinking (n=6), an intracorneal ring segment

(n=4), or a corneal penetrating transplant (n=1). As such, these eyes were excluded from analysis at the 48-month follow-up. Our primary outcome was the variation of visual, topographic and tomographic parameters following TE-ACXL. After undergoing TE-ACXL, there was a significant improvement in CDVA at 12 and at 48 months. However, there was also a significant increase in Kmean and D-index throughout follow-up and a significant decrease in PRC and PachyMin (only at 36 and 48 months for the latter). There were no intraoperative or postoperative complications regarding the procedure. Therefore, even though there was a significant improvement in CDVA, most eyes still presented a significant increase in Kmean and D-index, and a significant decrease in PRC and PachyMin after undergoing TE-ACXL, which raises questions about its effectiveness in halting progression in KC, especially considering that there are newer techniques to improve CXL's efficacy and effectiveness.[31] The questionable effectiveness of this technique may be related to the limited penetration of riboflavin in the corneal stroma,[32] the limited availability of oxygen,[33,34] and the lower UV-A transmission[35] in the TE-ACXL technique. These limitations are being addressed in more recent varitations of the technique, that include increasing the total UV-A energy to 7.2 J/cm^2,[36] as well as using pulsed light to increase the intrastromal concentration of oxygen,[37] or even using supplemental oxygen near the cornea, during the procedure.[33] These modifications may enhance the effectiveness of this technique, particularly in young patients with a high preoperative Kmean and decreased CDVA, such as the patients included in this study (baseline age of 20.90 ± 4.69 years, baseline Kmean of 48.66 ± 3.97 D, and baseline CDVA of 0.47 ± 0.36 logMAR units).

Since its first description by Wollensak *et al* in 2003, CXL has been widely used and proved to be a safe and effective method of corneal stabilization in keratoconus patients.[20,38] From the various treatment approaches to this condition, only CXL focuses on the disease's pathophysiology and natural history, aiming to slow down or cease its progression.[2,19,39,40] However, CXL is not devoid of adverse effects. As so, several modifications to the conventional protocol have been made to improve this procedure, with overall favourable results.[30] The TE-CXL procedure emerged as a solution to the problems related to the removal of the corneal epithelium, with several studies highlighting its faster healing, improved patient comfort, lower risk of corneal haze or infectious keratitis, and a better safety profile in advanced cases in which low corneal thickness would preclude treatment.[19,41,42] After the realization that the epithelium would act as a barrier and reduce the effects of riboflavin and UVA on the cornea, it became clear that maintaining it throughout the whole procedure would require methods to facilitate riboflavin diffusion, such as chemical enhancers (EDTA, Tris, and BAC, used in the present study, are examples of these substances) or iontophoresis.[21,43,44] Posteriorly, the A-CXL protocols were introduced to reduce illumination time by increasing intensity while maintaining the overall fluence, in accordance with the aforementioned Bunsen-Roscoe law.[45-48] Nonetheless, this law may not directly apply to CXL in living corneal tissue[49] and the higher irradiance required may lead to excessive oxygen consumption and therefore less availability, and less production of reactive oxygen species (ROS).[23,34] Furthermore, the corneal epithelium blocks about 20% of the UV illumination administered.[50] Combining these two modified protocols into TE-ACXL brings about both their advantages and disadvantages. Thus, studying its effect during the longest possible follow-up period is essential to determine if such a combination is effective in the treatment of progressive KC. Published evidence on TE-ACXL has increased significantly since its inception, reflecting the increasing interest in this method. Most studies acknowledge its safety and efficacy, albeit most of them agree that more long-term studies are required.[51-53]

There is an extremely relevant issue with TE-CXL, which is the wide variety of TE-CXL protocols and approaches currently used. There are many methodologies to increase corneal epithelial permeability to riboflavin (different chemical enhancers, iontophoresis), and multiple UVA irradiation protocols (18 mW/cm^2 irradiation for 5 minutes, 10 mW/cm^2 irradiation for 9 minutes, 6 mW/cm^2 for 15 minutes, 3 mW/cm^2 irradiation for 30 minutes, or even used pulsed protocols of irradiation of 45 mW/cm^2 for 5:20 min), though the total irradiation dose is generally similar (5.4 J/cm^2).[29] Naturally, with this lack of currently agreed upon TE-CXL protocol, drawing conclusions about the non-inferiority of TE-CXL when compared to epi-off CXL is difficult. Furthermore, there are still no RCTs that compare the long-term outcomes (at least 5 years) of TE-CXL and epi-off CXL.

We compared our results to other large prospective studies with a long follow-up (≥ 36 months) that used TE-ACXL: Al Fayez *et al*, who evaluated 36 patients with a baseline age of 24.8 ± 4.2 years, Kmean of 48.2 ± 3.6 D, and CDVA of 0.2 ± 0.2 logMAR units[54]; and, Henriquez *et al*, who evaluated 32 patients with a baseline age of 13.2 ± 2.6 years, Kmean 47.32 ± 2.78 D, and CDA of 0.19 ± 0.17 logMAR units).[55] In our study, we found a significant improvement in CDVA of 0.10 ± 0.29 logMAR units and 0.17 ± 0.33 logMAR units in BCVA at 12 and 48 months, respectively. Henriquez *et al* (who used a transepithelial riboflavin solution of 0.25% riboflavin, 1.0% phosphate hydroxypropyl methylcellulose, and 0.007% BAC, and irradiated the eyes for 5 minutes with an intensity of 18 mW/cm^2) reported a significant improvement in CDVA of 0.09 ± 0.17 logMAR units and 0.06 ± 0.19 logMAR units at 12 and 60 months postoperatively.[55] Al Fayez *et al* (who used a solution of tetracaine 1% with benzalkonium chloride 0.02% every 2 minutes, for 30 minutes, before starting no-dextran riboflavin drops, and irradiated the eyes for 30 minutes with an intensity of 3 mW/cm^2) reported a decrease in CDVA of 0.02 and 0.06 logMAR units at 12 and 36 months, respectively.[54] In our study, there were no significant differences in Kmax throughout follow-up, while Kmean increased significantly throughout follow-up (+0.64 ± 1.04 and +1.13 ± 2.00 at 12 and 48 months, respectively). Al Fayez *et al* reported a significant increase in Kmax of around +0.75 and +1.00D at 24 and 36 months, respectively, while Henriquez *et al* did not find significant differences in Kmax or Kmean at 12 or 60 months.[54,55]

In our study, PachyMin decreased significantly at 36 and 48 months (-8.50 ±15.93 and -7.83 ± 18.37 µm), while Henriquez *et al* reported no significant changes at 12 months and a significant increase of 7.33 ± 11.78 µm at 60 months.[55] In our study, there was a significant decrease in the PRC and a significant increase in the D-index throughout follow-up (from 12 to 48 months). None of the two aforementioned studies evaluated these parameters. Our study sample (baseline age of 20.90 ± 4.69 years, Kmean of 48.66 ± 3.97 D, and CDVA of 0.47 ± 0.36 logMAR units) was more similar to the one from Al Fayez *et al* than to the one from Henriquez *et al*.[54,55]

Our study limitations include its retrospective and non-randomized nature and the lack of a comparison epi-off CXL group. Furthermore, we did not include patients with very thin corneas (PachyMin < 380 µm), in order to study a more homogeneous population with progressive keratoconus. Nonetheless, some patients with progressive keratoconus and thin corneas also benefit from TE-ACXL, particularly with the use of hypoosmolar riboflavin, as has already been demonstrated in previous studies.[56,57] We believe that we have a relevant sample size of eyes that underwent TE-ACXL (when compared to other relevant studies that were published on this technique) and that our follow-up of 48 months allows for a reasonable evaluation of long-term outcomes of TE-ACXL. Nevertheless, the exclusion of 11 eyes that underwent additional procedures after 36 months could bias the results, as these cases may represent more severe or progressing disease. We opted to exclude these cases only from the 48-month comparison, since it would not be feasible to compare these eyes after they underwent additional procedures, but we kept these eyes in all the other analyses throughout follow-up, to limit the potential bias of excluding them altogether. There is still a significant need for RCTs that define the best TE-CXL protocol and that compare long-term outcomes of epi-off and TE-CXL. Only then will we be able to clearly define which cases should be treated with epi-off, and which cases should be treated with TE-CXL.

In conclusion, our results demonstrate that TE-ACXL was a safe treatment for eyes with progressive KC, increasing CDVA 4 years after the initial procedure. A significant proportion of eyes with progressive KC still presented significant variations in topographic, tomographic, and pachymetric parameters after being treated with TE-ACXL, requiring further procedures to halt disease progression. Nevertheless, future studies should focus on analyzing which patients benefit the most from TE-ACXl, as there is biological plausibility and clinical evidence that demonstrates that this is still a valuable technique in some cases of progressive keratoconus.

CONTRIBUTORSHIP STATEMENT / DECLARAÇÃO DE CONTRIBUIÇÃO

All authors contributed to the study's conception and design. Material preparation was performed by RVM, AMC, RM, LT, PNC, and JPC. Data collection and analysis were performed by RVM and MFR. The first draft of the manuscript was written byRVM, AMF, and JPC, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Todos os autores contribuíram para a conceção e desenho do estudo. A preparação do material foi efectuada por RVM, AMC, RM, LT, PNC e JPC. A recolha e análise de dados foram efectuadas por RVM e MFR. O primeiro rascunho do manuscrito foi escrito por RVM, AMF, e JPC, e todos os autores comentaram versões anteriores do manuscrito. Todos os autores leram e aprovaram o manuscrito final.

RESPONSABILIDADES ÉTICAS

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.

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Proteção de Pessoas e Animais: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pela Comissão de Ética responsável e de acordo com a Declaração de Helsínquia revista em 2013 e da Associação Médica Mundial.

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ETHICAL DISCLOSURES

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Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).

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REFERENCES

1. Mas Tur V, MacGregor C, Jayaswal R, O'Brart D, Maycock N. A review of keratoconus: Diagnosis, pathophysiology, and genetics. Surv Ophthalmol. 2017;62:770-83. doi: 10.1016/j.survophthal.2017.06.009.
2. Davidson AE, Hayes S, Hardcastle AJ, Tuft SJ. The patho-

genesis of keratoconus. Eye. 2014;28:189-95. doi: 10.1038/eye.2013.278.
3. Rabinowitz YS. Keratoconus. Surv Ophthalmol. 1998;42:297-319.
4. Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS. Keratoconus: a review. Cont Lens Anterior Eye. 2010;33:157-66; quiz 205. doi: 10.1016/j.clae.2010.04.006.
5. Godefrooij DA, de Wit GA, Uiterwaal CS, Imhof SM, Wisse RP. Age-specific Incidence and Prevalence of Keratoconus: A Nationwide Registration Study. Am J Ophthalmol. 2017;175:169-72. doi: 10.1016/j.ajo.2016.12.015.
6. Torres Netto EA, Al-Otaibi WM, Hafezi NL, Kling S, Al-Farhan HM, Randleman JB, et al. Prevalence of keratoconus in paediatric patients in Riyadh, Saudi Arabia. Br J Ophthalmol. 2018;102:1436-41. doi: 10.1136/bjophthalmol-2017-311391.
7. Ambrósio R Jr, Lopes BT, Faria-Correia F, Salomão MQ, Bühren J, Roberts CJ, et al. Integration of scheimpflug-based corneal tomography and biomechanical assessments for enhancing ectasia detection. J Refract Surg. 2017;33:434-43. doi: 10.3928/1081597X-20170426-02.
8. Duncan JK, Belin MW, Borgstrom M. Assessing progression of keratoconus: novel tomographic determinants. Eye Vis. 2016;3:6. doi: 10.1186/s40662-016-0038-6.
9. Jay JM, Akilesh G, Hans RV, Charles NJ. Progression of keratoconus in children and adolescents. Br J Ophthalmol. 2023;107:176-180. doi: 10.1136/bjophthalmol-2020-316481.
10. Gomes JA, Tan D, Rapuano CJ, Belin MW, Ambrósio R, Jr., Guell JL, et al. Global consensus on keratoconus and ectatic diseases. Cornea. 2015;34:359-69. doi: 10.1097/ICO.0000000000000408.
11. Ribeiro M, Barbosa C, Correia P, Torrão L, Neves Cardoso P, Moreira R, et al. Best fit sphere back and adjusted maximum elevation of corneal back surface as novel predictors of keratoconus progression. Clin Ophthalmol. 2022;16:4239-48. doi: 10.2147/OPTH.S388614.
12. Cunha AM, Correia PJ, Alves H, Torrão L, Moreira R, Falcão-Reis F, et al. Keratoconus enlargement as a predictor of keratoconus progression. Sci Rep. 2021;11:21079. doi: 10.1038/s41598-021-00649-0.
13. Jiménez-García M, Kreps EO, S ND, Koppen C, Rozema JJ. Determining the most suitable tomography-based parameters to describe progression in keratoconus. the retrospective digital computer analysis of keratoconus evolution project. Eye Contact Lens. 2021;47:486-93. doi: 10.1097/ICL.0000000000000800.
14. Gustafsson I, Bergström A, Cardiakides A, Ivarsen A, Hjortdal J. The Interday Repeatability of Parameters for the Assessment of Progressive Disease in Subjects With Less Advanced Keratoconus. Am J Ophthalmol. 2021;225:38-46. doi: 10.1016/j.ajo.2020.12.028.
15. Martínez-Abad A, Piñero DP. New perspectives on the detection and progression of keratoconus. J Cataract Refract Surg. 2017;43:1213-27. doi: 10.1016/j.jcrs.2017.07.021.
16. Shajari M, Steinwender G, Herrmann K, Kubiak KB, Pavlovic I, Plawetzki E, et al. Evaluation of keratoconus progression. Br J Ophthalmol. 2019;103:551-7. doi: 10.1136/bjophthalmol-2017-311651.
17. Deshmukh R, Ong ZZ, Rampat R, Alió Del Barrio JL, Barua A, Ang M, et al. Management of keratoconus: an updated review. Front Med. 2023;10:1212314. doi: 10.3389/fmed.2023.1212314.
18. Valera-Cornejo DA, Vega-Estrada A, Alio JL. Invasive pharmacology outcomes with different corneal cross-linking protocols: a review. J Ocul Pharmacol Ther. 2019;35:475-90. doi: 10.1089/jop.2018.0144.
19. Subasinghe SK, Ogbuehi KC, Dias GJ. Current perspectives on corneal collagen crosslinking (CXL). Graefes Arch Clin Exp Ophthalmol. 2018;256:1363-84. doi: 10.1007/s00417-018-3966-0.
20. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol. 2003;135:620-7. doi: 10.1016/s0002-9394(02)02220-1.
21. Heikal MA, Soliman TT, Fayed A, Hamed AM. Efficacy of transepithelial corneal collagen crosslinking for keratoconus: 12-month follow-up. Clin Ophthalmol. 2017;11:767-71. doi: 10.2147/OPTH.S129037.
22. Beckman KA, Gupta PK, Farid M, Berdahl JP, Yeu E, Ayres B, et al. Corneal crosslinking: Current protocols and clinical approach. J Cataract Refract Surg. 2019;45:1670-9. doi: 10.1016/j.jcrs.2019.06.027.
23. O'Brart DP. Corneal collagen crosslinking for corneal ectasias: a review. Eur J Ophthalmol. 2017;27:253-69. doi: 10.5301/ejo.5000916.
24. Medeiros CS, Giacomin NT, Bueno RL, Ghanem RC, Moraes HV, Jr., Santhiago MR. Accelerated corneal collagen crosslinking: Technique, efficacy, safety, and applications. J Cataract Refract Surg. 2016;42:1826-35. doi: 10.1016/j.jcrs.2016.11.028.
25. Cunha AM, Sardinha T, Torrão L, Moreira R, Falcão-Reis F, Pinheiro-Costa J. Transepithelial accelerated corneal collagen cross-linking: two-year results. Clin Ophthalmol. 2020;14:2329-37. doi: 10.2147/OPTH.S252940.
26. Li W, Wang B. Efficacy and safety of transepithelial corneal collagen crosslinking surgery versus standard corneal collagen crosslinking surgery for keratoconus: a meta-analysis of randomized controlled trials. BMC Ophthalmol. 2017;17:262. doi: 10.1186/s12886-017-0657-2.
27. Ng AL, Chan TC, Lai JS, Cheng AC. Comparison of the central and peripheral corneal stromal demarcation line depth in conventional versus accelerated collagen cross-linking. Cornea. 2015;34:1432-6. doi: 10.1097/ICO.0000000000000626.
28. Shalchi Z, Wang X, Nanavaty MA. Safety and efficacy of epithelium removal and transepithelial corneal collagen crosslinking for keratoconus. Eye. 2015;29:15-29. doi: 10.1038/eye.2014.230.
29. D'Oria F, Palazón A, Alio JL. Corneal collagen cross-linking epithelium-on vs. epithelium-off: a systematic review and meta-analysis. Eye Vis. 2021;8:34. doi: 10.1186/s40662-021-00256-0.
30. Cifariello F, Minicucci M, Di Renzo F, Di Taranto D, Coclite G, Zaccaria S, et al. Epi-Off versus Epi-On corneal collagen cross-linking in keratoconus patients: a comparative study through 2-year follow-up. J Ophthalmol. 2018;2018:4947983. doi: 10.1155/2018/4947983.
31. Wu D, Lim DK, Lim BXH, Wong N, Hafezi F, Manotosh R, et al. Corneal Cross-Linking: The Evolution of Treatment for Corneal Diseases. Front Pharmacol. 2021;12:686630. doi: 10.3389/fphar.2021.686630.
32. Mastropasqua L, Nubile M, Calienno R, Mattei PA, Pedrotti E, Salgari N, et al. Corneal cross-linking: intrastromal riboflavin concentration in iontophoresis-assisted imbibition versus traditional and transepithelial techniques. Am J Ophthalmol. 2014;157:623-30.e1. doi: 10.1016/j.ajo.2013.11.018.
33. Hill J, Liu C, Deardorff P, Tavakol B, Eddington W, Thompson V, et al. Optimization of Oxygen Dynamics, UV-A Delivery, and Drug Formulation for Accelerated Epi-On Corneal Crosslinking. Curr Eye Res. 2020;45:450-8. doi: 10.1080/02713683.2019.1669663.
34. Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F. The Biomechanical Effect of Corneal Collagen Cross-Linking (CXL) With Riboflavin and UV-A is Oxygen Dependent. Transl Vis Sci Technol. 2013;2:6. doi: 10.1167/tvst.2.7.6.
35. Podskochy A. Protective role of corneal epithelium against ultraviolet radiation damage. Acta Ophthalmol Scand.

2004;82:714-7. doi: 10.1111/j.1600-0420.2004.00369.x.
36. Bilgihan K, Yesilirmak N, Altay Y, Yuvarlak A, Ozdemir HB. Conventional corneal collagen cross-linking versus transepithelial diluted alcohol and iontophoresis-assisted corneal cross-linking in progressive keratoconus. Cornea. 2017;36:1492-7. doi: 10.1097/ICO.0000000000001383.
37. Mazzotta C, Bagaglia SA, Sgheri A, Di Maggio A, Fruschelli M, Romani A, et al. Iontophoresis Corneal Cross-linking With Enhanced Fluence and Pulsed UV-A Light: 3-Year Clinical Results. J Refract Surg. 2020;36:286-92. doi: 10.3928/1081597X-20200406-02.
38. Hersh PS, Stulting RD, Muller D, Durrie DS, Rajpal RK. United States Multicenter Clinical Trial of Corneal Collagen Crosslinking for Keratoconus Treatment. Ophthalmology. 2017;124:1259-70. doi: 10.1016/j.ophtha.2017.03.052.
39. Gordon MO, Steger-May K, Szczotka-Flynn L, Riley C, Joslin CE, Weissman BA, et al. Baseline factors predictive of incident penetrating keratoplasty in keratoconus. Am J Ophthalmol. 2006;142:923-30. doi: 10.1016/j.ajo.2006.07.026.
40. Matthaei M, Sandhaeger H, Hermel M, Adler W, Jun AS, Cursiefen C, et al. Changing indications in penetrating keratoplasty: a systematic review of 34 years of global reporting. 2017;101:1387-99. doi: 10.1097/TP.0000000000001281.
41. Hersh PS, Lai MJ, Gelles JD, Lesniak SP. Transepithelial corneal crosslinking for keratoconus. J Cataract Refract Surg. 2018;44:313-22. doi: 10.1016/j.jcrs.2017.12.022.
42. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. J Cataract Refract Surg. 2009;35:540-6. doi: 10.1016/j.jcrs.2008.11.036.
43. Lombardo M, Giannini D, Lombardo G, Serrao S. Randomized Controlled Trial Comparing Transepithelial Corneal Cross-linking Using Iontophoresis with the Dresden Protocol in Progressive Keratoconus. Ophthalmology. 2017;124:804-12. doi: 10.1016/j.ophtha.2017.01.040.
44. Lombardo M, Serrao S, Lombardo G, Schiano-Lomoriello D. Two-year outcomes of a randomized controlled trial of transepithelial corneal crosslinking with iontophoresis for keratoconus. J Cataract Refract Surg. 2019;45:992-1000. doi: 10.1016/j.jcrs.2019.01.026.
45. Sadoughi MM, Einollahi B, Baradaran-Rafii A, Roshandel D, Hasani H, Nazeri M. Accelerated versus conventional corneal collagen cross-linking in patients with keratoconus: an intrapatient comparative study. Int Ophthalmol. 2018;38:67-74. doi: 10.1007/s10792-016-0423-0.
46. Tomita M, Mita M, Huseynova T. Accelerated versus conventional corneal collagen crosslinking. J Cataract Refract Surg. 2014;40:1013-20. doi: 10.1016/j.jcrs.2013.12.012.
47. Shetty R, Pahuja NK, Nuijts RM, Ajani A, Jayadev C, Sharma C, et al. Current protocols of corneal collagen cross-linking: visual, refractive, and tomographic outcomes. Am J Ophthalmol. 2015;160:243-9. doi: 10.1016/j.ajo.2015.05.019.
48. Waszczykowska A, Jurowski P. Two-year accelerated corneal cross-linking outcome in patients with progressive keratoconus. Biomed Res Int. 2015;2015:325157. doi: 10.1155/2015/325157.
49. Spadea L, Tonti E, Vingolo EM. Corneal stromal demarcation line after collagen cross-linking in corneal ectatic diseases: a review of the literature. Clin Ophthalmol. 2016;10:1803-10. doi: 10.2147/OPTH.S117372.
50. D'Oria F, Puzo P, Incandela C, Sborgia A, Gigliola S, Boscia F, et al. Evaluation of demarcation line after epithelium-off iontophoresis corneal collagen cross-linking for progressive keratoconus. J Clin Med. 2021;10:3295. doi: 10.3390/jcm10153295.
51. Sun L, Li M, Zhang X, Tian M, Han T, Zhao J, et al. Transepithelial accelerated corneal collagen cross-linking with higher oxygen availability for keratoconus: 1-year results. Int Ophthalmol. 2018;38:2509-17. doi: 10.1007/s10792-017-0762-5.
52. Tian M, Jian W, Zhang X, Sun L, Zhou X. Three-year follow-up of accelerated transepithelial corneal cross-linking for progressive paediatric keratoconus. Br J Ophthalmol. 2020;104:1608-12. doi: 10.1136/bjophthalmol-2019-315260.
53. Madeira C, Vasques A, Beato J, Godinho G, Torrão L, Falcão M, et al. Transepithelial accelerated versus conventional corneal collagen crosslinking in patients with keratoconus: a comparative study. Clin Ophthalmol. 2019;13:445-52. doi: 10.2147/OPTH.S189183.
54. Al Fayez MF, Alfayez S, Alfayez Y. Transepithelial Versus Epithelium-Off Corneal Collagen Cross-Linking for Progressive Keratoconus: A Prospective Randomized Controlled Trial. Cornea. 2015;34:S53-6. doi: 10.1097/ICO.0000000000000547.
55. Henriquez MA, Hernandez-Sahagun G, Camargo J, Izquierdo L, Jr. Accelerated Epi-On Versus Standard Epi-Off Corneal Collagen Cross-Linking for Progressive Keratoconus in Pediatric Patients: Five Years of Follow-Up. Cornea. 2020;39:1493-8. doi: 10.1097/ICO.0000000000002463.
56. Gu S, Fan Z, Wang L, Tao X, Zhang Y, Mu G. Corneal collagen cross-linking with hypoosmolar riboflavin solution in keratoconic corneas. Biomed Res Int. 2014;2014:754182. doi: 10.1155/2014/754182.
57. Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refract Surg. 2009;35:621-4. doi: 10.1016/j.jcrs.2008.10.060.

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