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Effect of high and low molecular weight sodium hyaluronic acid eye drops on corneal recovery after crosslinking in keratoconus patients

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ABSTRACT

Objective To assess the impact of eye drops containing high molecular weight hyaluronic acid (HMW-HA) and low molecular weight hyaluronic acid (LMW-HA) on corneal nerve regeneration, dendritic cell (DC) density, corneal sensitivity (CS) and ocular surface parameters in patients with keratoconus following corneal crosslinking (CXL).

Methods and analysis 63 eyes of 55 patients with keratoconus were randomised to receive eye drops containing HMW-HA (n: 20) for 12 months, LMW-HA (n: 23) for 12 months and polyvinyl alcohol (n: 20) until closure of the epithelial defect in the control group after CXL. Subbasal nerve plexus (SNP) was imaged with corneal confocal microscopy and quantified with ACCMetrics. DC density was calculated with Image J. Ocular Surface Disease Index (OSDI) questionnaire and non-invasive break-up time (NI-TBUT) were evaluated. All measurements were performed before CXL and 1, 3, 6 and 12 months postsurgery.

Results At 6 months post-CXL, SNP reached to its preoperative. CS was higher in the HMW-HA groups compared with the other two groups in the 3rd and 6th month post-CXL. DC density was higher in the LMW-HA group compared with the HMW-HA group in the postoperative 3rd month. OSDI were higher in the control group compared with both the LMW-HA and HMW-HA groups at postoperative 3rd and 6th months. NI-TBUT was lower in the control group in the 6th and 12th months compared with the other groups.

Conclusions The use of artificial tear drops containing HMW-HA may have a therapeutic effect to promote corneal nerve regrowth and support faster functional recovery after CXL.

Trial registration number NCT06243991.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The molecular weight of the hyaluronic acid molecule determines its physical and physiological properties. However, clinical studies evaluating the effects of these properties on the ocular surface based on molecular weight are insufficient.

WHAT THIS STUDY ADDS

⇒ Previous literature has extensively covered the anti-inflammatory effects of high molecular weight hyaluronic acid in patients with dry eye disease while our study specifically investigates the impact on nerve regeneration after corneal crosslinking, comparing it with the low molecular weight form. The findings demonstrated that high molecular weight hyaluronic acid was more effective in corneal recovery.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings of this study could influence clinical practices by guiding the selection of sodium hyaluronic acid drops based on molecular weight for postoperative care.

INTRODUCTION

In recent years, corneal crosslinking (CXL) has emerged as a revolutionary therapeutic modality, offering a promising avenue for the management of progressive corneal diseases.[1] As evidenced by extensive long-term clinical studies, it has been understood that CXL is a proven and safe procedure for halting the progression of keratoconus.[2–4] Corneal confocal microscopy studies (CCM) can be used in vivo to better visualise and understand the morphological changes induced by CXL.[5–7] Notably, such studies revealed that the removal of the epithelium and exposure to ultraviolet A (UVA) lead to an immediate loss of the subbasal nerve plexus (SNP), followed by gradual regeneration, with the nerve plexus regaining its preoperative morphology within 6 months to 1-year post-treatment.[7–9] Corneal nerves assume a critical role in protective mechanisms such as blinking and tearing reflexes, along with maintaining the homeostasis of the ocular surface.[10] Furthermore, corneal cells actively contribute to the growth and function of corneal nerves by producing various trophic and growth factors.[11] Consequently, the intricate interactions between corneal cells and nerves are indispensable for the regenerative processes in a wounded cornea.

Hyaluronic acid (HA) belongs to the glycosaminoglycan family, a significant component

of the extracellular matrix, playing a crucial role in tissue regeneration processes.[12] Its characteristics and functions in biology differ according to molecular size. Based on its physiological properties, high molecular weight HA (HMW-HA) demonstrates anti-inflammatory properties and contributes to wound healing through receptor interactions.[12] In contrast, low molecular weight HA (LMW-HA) functions as a potent pro-inflammatory molecule.[12] Additionally, HMW-HA is recognised for its regulatory role in the proliferation, differentiation and migration of nerve cells.[13]

Various experimental and clinical studies have been conducted to assess the biological effects of HA at different molecular weights.[14–16] Kojima and colleagues established a dry eye stress animal model to investigate the effects of HMW-HA (Comfort Shield, i.com medical GmbH, Munich, Germany), LMW-HA (Thealose Duo, Thea, Clermont-Ferrand, France) and diquafasol sodium (DQ) eye drops on tear film stability.[14] Additionally, the anti-inflammatory effect of HMW-HA was evaluated by examining tear volume and Muc5Ac mRNA expression to determine its protective role against T cell-mediated damage to the lacrimal gland and goblet cells.[14] The study revealed that HMW-HA had better tear film stability as determined by tear break-up time (TBUT).[14] Moreover, HMW-HA eye drops increased tear volume and Muc5RNA expression more than DQ eye drops, even though it is a secretagogue.[14] In addition, inflammatory dendritic cell (DC) density was lower in the HMW-HA group.[14] This animal study emphasised the importance of evaluating the differences in the effects of HA based on its molecular weight.

Recent clinical research on humans has indicated that HMW-HA eye drops contribute to the recovery of corneal nerves in patients with dry eyes. However, in this study, the anti-inflammatory effect of HMW-HA has not been evaluated.[17] Previous studies investigating the use of sodium hyaluronate after CXL have primarily focused on epithelial healing.[18–20] While one study has explored its effect on corneal nerve regeneration, the molecular weight was not specified.[21] To the best of our knowledge, there is no clinical study comparing the effects of HMW-HA and LMW-HA eye drops after CXL. The primary outcome of this study was to assess corneal nerve regeneration following CXL with different molecular weights of sodium hyaluronate. Secondary outcomes included ocular surface parameters, DC density and corneal sensitivity (CS) after CXL.

METHODS

The registration information for this human clinical trial is available at http://www.clinicaltrials.gov (identifier NCT06243991). The recruitment period spanned from 1 March 2021 to 1 December 2022.

Subjects and examination

This study assessed individuals aged 18 and above diagnosed with keratoconus and scheduled for epithelium-off CXL. Exclusion criteria were corneal thickness below 400 µm, pregnancy, breastfeeding, use of topical or systemic medications, presence of eye diseases other than keratoconus, systemic diseases, active atopy or allergies, contact lens usage and a history of ocular surgery. A total of 63 eyes from 55 keratoconus patients were randomly assigned using computer-generated randomisation (www.random.org/integers) into three groups: 20 eyes in the HMW-HA group, 23 eyes in the LMW-HA group and 20 eyes in the control group without the administration of artificial tears (online supplemental figure 1).[22] Post-CXL, the HMW-HA group received topical HMW-HA (Comfort Shield, i.com medical GmbH) three times per day for 12 months, the LMW-HA group received topical LMW-HA (Thealose Duo, Thea) three times per day for 12 months. The control group received artificial tears containing topical polyvinyl alcohol (Refresh, Allergan, Dublin, Ireland) three times per day until closure of the epithelial defect, after which it was discontinued. All participants underwent accelerated epithelium-off CXL for 10 min with 9 mW/cm² UVA irradiation. The postoperative standard treatment regimen included topical moxifloxacin (0.5% Vigamox, Alcon, USA) for 1 week, topical dexamethasone (0.1% Dexasine-SE, Kaysersberg Pharmaceuticals, France) for 1 week after epithelial closure, followed by topical loteprednol 0.5% (Lotemax, Bausch & Lomb, USA) for 3 weeks.

Uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA) and manifest spherical equivalent (SE) were recorded at all visits. In addition, the following parameters were assessed and tests performed, carried out in the same order: Ocular Surface Disease Index (OSDI) questionnaire, non-invasive TBUT (NI-TBUT), corneal tomography (Pentacam, OCULUS, Wetzlar, Germany), CS, corneal fluorescein staining and CCM imaging. All these assessments were performed preoperatively and 1, 3, 6 and 12 months postsurgery.

Ocular surface evaluation

The Turkish validated version of the OSDI, a questionnaire assessing clinical symptoms related to ocular surface disease, was employed.[23] The OSDI questionnaire consists of a total of 12 questions categorised into three subscales as follows: ocular symptoms, vision-related function and environmental triggers. Each patient is asked to rate the symptoms on a 5-point scale ranging from never (0 score) to always (4 score) for every question in the questionnaire. The fourth and fifth questions in the first section, concerning blurred vision and reduced vision symptoms, were excluded from the questionnaire as these symptoms may already be present in patients with keratoconus disease.[24] The total OSDI score was calculated according to the formula: OSDI=[(sum of scores for all questions answered)×100]/[(total number of questions answered)×4].

NI-TBUT was assessed using a Sirius Scheimpflug camera (CSO, Florence, Italy) and the device automatically provided the average NI-TBUT value.

Ocular surface staining was evaluated using sterile fluorescein strips. Staging was performed according to the Oxford scheme.[25]

CCM and SNP analysis

CCM was performed using the Heidelberg Retinal Tomograph 3 with the Rostock Cornea Module (HRT3-RCM, Heidelberg Engineering GmbH, Germany) under topical anaesthesia. A viscous gel (Viscotears, Novartis Pharmaceuticals UK) served as a coupling agent between the cornea and the applanation cap. The subjects were instructed to focus on the fixation light with the unexamined eye to ensure proper positioning. Five high-quality images of the SNP were selected and analysed using the automated tracing of nerve fibres programme (ACCMetrics, M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, England). The following parameters were obtained:

1. Corneal nerve fibre density (CNFD), the total number of nerves per mm^2 in a frame divided by the image area. (area: 0.16 mm^2).
2. Corneal nerve branch density (CNBD), the number of branches emanating from major nerve trunks per mm^2 in a frame divided by the image area. (area: 0.16 mm^2).
3. Corneal nerve fibre length (CNFL), the total length of all nerve fibres and branches per mm^2 in a frame divided by the image area. (area: 0.16 mm^2).
4. Corneal nerve total branch density (CTBD), the total number of branches per mm^2 in a frame divided by the image area (area: 0.16 mm^2).

In addition, the density of DC was calculated using ImageJ software (Image V.1.31; National Institutes of Health). The statistical analysis used the average of the five values for each parameter.

Corneal sensitivity

CS was evaluated using a Cochet-Bonnet esthesiometer (Luneau Ophtalmologue, Chartres, France), comprising a nylon filament measuring 60 mm in length and 0.12 mm in diameter. Participants were instructed to maintain a forward gaze while the esthesiometer gently made perpendicular contact. The procedure involved gradually decreasing the filament length in 5 mm increments, starting from 60 mm, until the initial response from the subject was detected.

Statistical analysis

The SPSS statistical software (V.26.0, IBM, Armonk, NY, USA) was employed for data analysis. Descriptive statistics are presented as mean±SD or median and 95% CIs. Categorical data were assessed using the χ^2 test. Normality of data distribution was determined through the Kolmogorov-Smirnov test and histogram graphs. Comparisons among more than two independent groups were conducted using the Kruskal-Wallis test or one-way analysis of variance (ANOVA), depending on the normality of the distribution. Post hoc analyses included Kruskal-Wallis, pairwise comparison with Bonferroni correction, or ANOVA with Bonferroni-Dunn correction. For analysis of multiple dependent variables over time, repeated measures ANOVA or the Friedman test was employed. Post hoc analyses for repeated measures involved repeated measures ANOVA with Bonferroni-Dunn correction or the Friedman test, pairwise comparison, with Bonferroni correction. A p value below 0.05 was considered statistically significant.

The GPower V.3.1 software was used for power analysis. In the study conducted by Sabur and Acar, preoperative CNFD values (10.73±3.52) were compared with postoperative 6-month CNFD values (7.02±1.42) after CXL (effect size: 1.209).[21] With an alpha value of 0.05 and a power value of 0.95 calculated for the Wilcoxon signed-rank test for paired ordinal variables, it was determined that a minimum of 10 patients per group would be required.

Patient and public involvement

Patients and the public were not involved in the design, conduct, reporting or dissemination of this research.

Table 1 Demographic and clinical characteristics of the patients

	Control	LMW-HA	HMW-HA	P value
Age	22.30±2.02	22.91±3.57	23.05±2.64	0.699*
Male:Female	11/8	16/6	13/4	0.150†
Eyes (n)	20	23	20	
Keratoconus stage (n) ‡				0.501†
Stage I	13 (65.0%)	10 (25.0%)	9 (4.0%)	
Stage II	5 (25.0%)	11 (47.8%)	10 (50.8%)	
Stage III	2 (10.0%)	2 (8.7%)	1 (5.0%)	

*One-way analysis of variance test.
†χ^2 test.
‡Based on Amsler-Krumeich classification.
HMW-HA, high molecular weight hyaluronic acid; LMW-HA, low molecular weight hyaluronic acid.

Table 2 Changes in tomographic parameters between preoperative and postoperative 12th month

	Control	LMW-HA	HMW-HA	P value*
ΔK1 (D)	−0.47±0.57	−0.28±0.64	−0.44±0.60	0.720
ΔK2 (D)	−0.41±0.57	−0.57±0.59	−0.50±0.71	0.423
ΔKmax (D)	−0.88±1.15	−1.01±0.89	−1.00±1.06	0.860
ΔKmean (D)	−0.45±0.53	−0.42±0.46	−0.47±0.47	0.995
ΔTCT (µm)	2.30±10.85	1.13±14.21	−3.76±14.96	0.569

*Kruskal-Wallis.
D, Diopter; HMW-HA, high molecular weight hyaluronic acid; LMW-HA, low molecular weight hyaluronic acid; TCT, thinnest corneal thickness.

RESULTS

In this study, a total of 55 patients were enrolled. Table 1 summarises the characteristics of the patients.

Visual acuity, refractive and tomographic findings

The preoperative manifest SE, UCVA, BCVA and tomography findings, including thinnest corneal thickness, K1, K2, Kmean and Kmax, did not show statistically significant differences between the groups (p>0.05). SE, UCVA and BCVA remained comparable among the groups at all follow-ups (p>0.05).

A decrease in both Kmax and Kmean was observed in the HMW-HA group, whereas only Kmax decreased in the LMW-HA group at 12 months postoperatively compared with preoperative values (online supplemental table S1). However, the changes in tomographic findings, expressed as the difference between postoperative 12th month and preoperative measurements, were similar in all groups (table 2).

Ocular surface parameters

In all eyes, the Oxford scheme grading remained at grade '0' during both preoperative and postoperative follow-ups. Preoperative values for OSDI and NI-TBUT showed no significant differences between the groups (online supplemental table S2). However, the OSDI score was higher in the control group compared with both LMW-HA and HMW-HA groups at the 3rd, 6th and 12th months post-CXL (p<0.001). In both LMW-HA and HMW-HA groups, the OSDI score remained lower than the preoperative values at the 3rd, 6th and 12th months post-CXL (p<0.001). NI-TBUT was lower in the control group compared with the HMW-HA and LMW-HA groups at postoperative 6th months (p=0.014, p=0.001, respectively) and 12th months (p=0.005, p=0.037, respectively), with no significant difference observed between the HMW-HA and LMW-HA groups (p=1.000). NI-TBUT values showed no significant difference over time in any of the groups (p>0.05).

SNP analysis

The preoperative SNP analysis did not show significant differences between the groups in any of the parameters (figure 1). In the HMW-HA group, CNFD and CNBD values were higher in the 3rd and 6th postoperative months, whereas CNFL and CTBD were higher in the postoperative 6th month compared with the control and LMW-HA groups (figure 1). CNFD, CNFL, CNBD and CTBD were similar between groups at the postoperative 12th month (figure 1). All parameters decreased at postoperative 1st and 3rd months compared with its baseline in all groups and could not reach preoperative values at 6 months in control and LMW-HA groups (p<0.05) (online supplemental table S3). However, no differences were found between preoperative and postoperative 6th month SNP parameters in the HMW-HA group (CNFD; p=0.116, CNFL; p=0.179, CNBD; 0.187, CTBD; p=0.258) (online supplemental table S3). CNFD, CNFL, CNBD and CTBD recovered to their preoperative values in all groups at postoperative 12th month (p>0.05) (figure 2).

DC density

Preoperative DC densities were comparable across all groups (figure 3). In the postoperative 3rd month, lower DC density was observed in the HMW-HA group compared with the LMW-HA group (p=0.043). In the LMW-HA group, there was an increase in DC density at postoperative 3 months compared with the baseline (p=0.003). No significant differences were found between the groups in DC density at postoperative 6 and 12 months (figure 3).

Corneal sensitivity

In all eyes (n=63), preoperative CS was 60 mm. The mean CS in the HMW-HA group was greater compared with the control and LMW-HA groups at 1st, 3rd and 6th months postoperatively (p<0.001). CS in the HMW-HA group reached its baseline at postoperative 3rd month (56.25±4.83 mm), while in the LMW-HA group, it returned to preoperative levels at 6-month post-CXL (53.94±5.15 mm) (p=0.066, p=0.079, respectively). However, in the control group, CS remained significantly lower at postoperative 3rd (42.63±3.86 mm) and 6th months (47.63±4.82 mm) (p<0.001, p=0.010, respectively). CS reached preoperative levels in all groups at the 12th postoperative month (p=1.000).

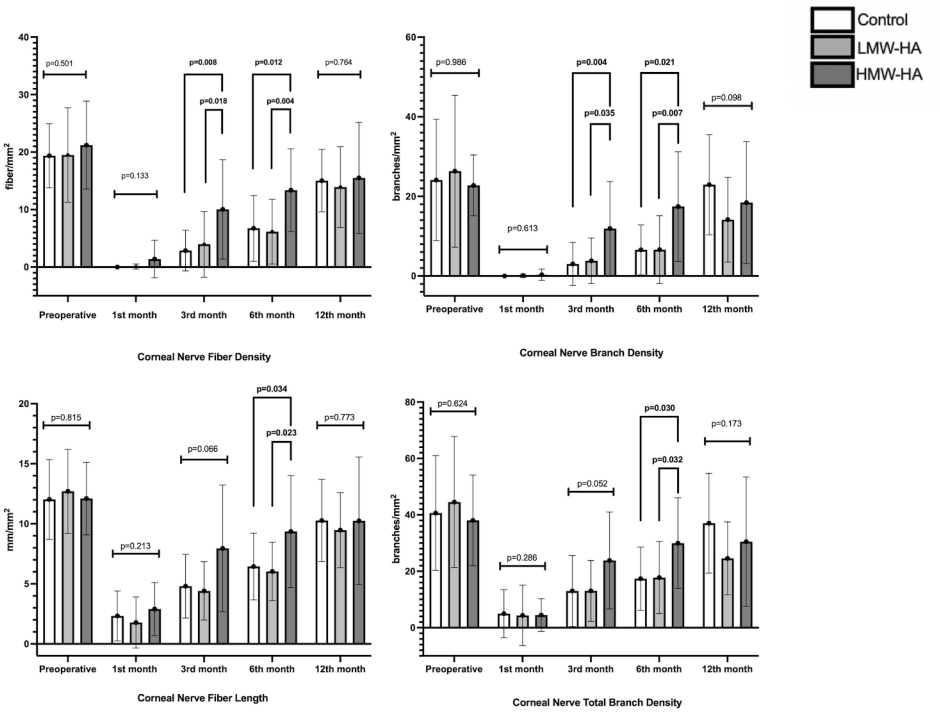

Figure 1 Comparison of subbasal nerve fibre parameters at follow-ups between groups. Mean values and SD are shown. *Kruskal-Wallis test, p values in bold are statistically significant. HMW-HA, high molecular weight hyaluronic acid; LMW-HA, low molecular weight hyaluronic acid.

DISCUSSION

This study aimed to investigate the differences in the clinical effects of HA, which exhibits distinct physicochemical properties at different molecular weights. We observed that the application of eye drops with HMW-HA supported faster nerve regeneration and faster recovery of CS, while improving other ocular surface parameters after CXL as compared with those with LMW-HA. In the multicentre study conducted by van Setten *et al*, the addition of HMW-HA eye drops to the treatment of patients with severe dry eye disease resulted in significant improvement in SNP, as observed with CCM.[17]

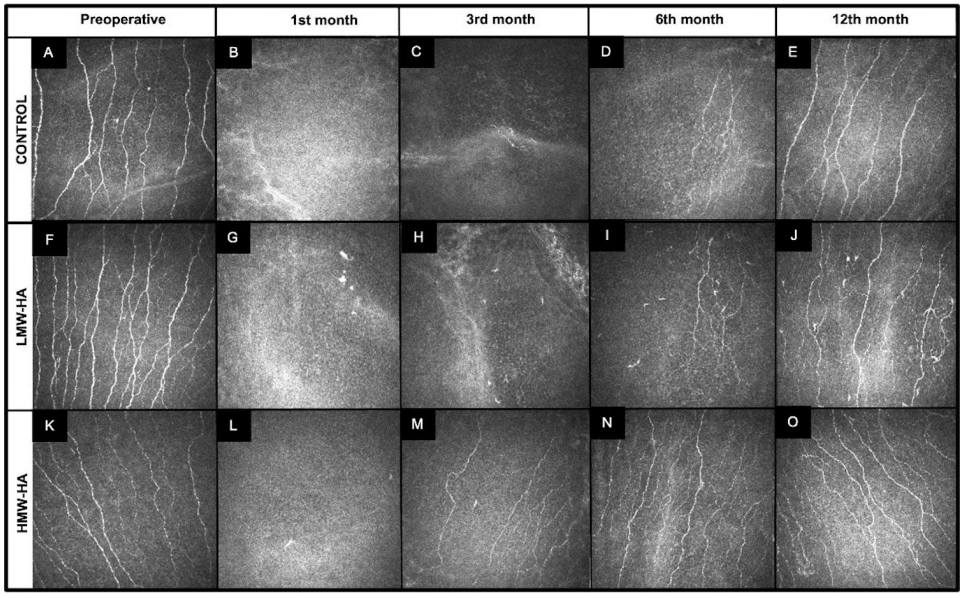

Figure 2 Representative in vivo confocal microscopy images of subbasal nerve plexus before and after CXL. (A–E) Control group. (F–J) LMW-HA group. (K–O) HMW-HA group. Preoperative image of subbasal nerves before CXL (A, F, K). Postoperative images; 1 month (B, G, L), 3 months (C, H, M), 6 months (D, I, N) and 12 months (E, J, O) after CXL. CXL, corneal crosslinking; HMW-HA, high molecular weight hyaluronic acid; LMW-HA, low molecular weight hyaluronic acid.

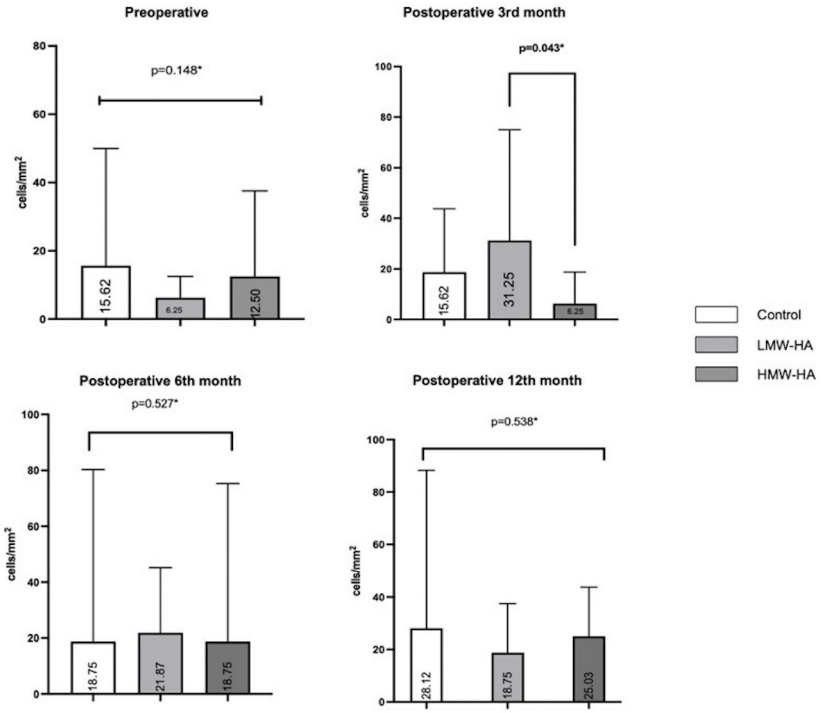

Figure 3 Comparisons of dendritic cell density in groups at follow-ups. Median values and 95% CIs are shown. *Kruskal Wallis test, p values in bold are statistically significant. HMW-HA, high molecular weight hyaluronic acid; LMW-HA, low molecular weight hyaluronic acid.

Moreover, the patients in the study experienced symptomatic relief, with less discomfort and pain.[26] These findings suggested that topically applied HMW-HA may have a neurotrophic effect on corneal nerves. However, the observed effect on nerve regeneration might be explained by the anti-inflammatory properties of HMW-HA, which could potentially address the underlying inflammatory pathology in dry eye disease. However, this study does not specifically assess a parameter that evaluates the anti-inflammatory effect. In our study, we compared DC density to assess inflammation. We observed an increase in DC density in the postoperative 3rd month, particularly in the LMW-HA group, known for its pro-inflammatory properties. Additionally, at the postoperative 3rd month, DC density in the HMW-HA group was lower compared with the LMW-HA group. This may be regarded as the clinical manifestation of HA, demonstrating pro-inflammatory effects in low molecular weight and anti-inflammatory effects in high molecular weight, but to confirm this, examining other inflammatory markers would be more conclusive. Moreover, nerve regeneration and inflammation are intertwined, such that an increase in DC could reflect an effect of inflammation and/or nerve regeneration.[27]

A recent study by Sabur and Acar compared the effect of eye drops containing only HA and a combination of HA with dexpanthenol (HA/DP) on corneal epithelial healing and corneal microstructural changes after CXL.[21] CCM revealed a faster regeneration of the SNP in HA/DP treated eyes when compared with eyes treated with only 0.15% HA (Eyestil, Sifi, Italy). However, unlike our study, Sabur and Acar assessed nerve density only and the nerve density remained lower at the postoperative 6th month compared with baseline in both groups. In this study, the information about sodium HA eye drops only includes their concentration, with no mention of their molecular weight. Indeed, manufacturers typically provide information regarding the concentration of HA without specifying its molecular weight, which makes it very difficult to correlate the performance of different products reported in the literature. Therefore, in vitro studies assessing the physicochemical properties of HA may help us better evaluate and understand the effects of different molecular weights of HA.[28]

Measurement of CS provides a functional assessment of corneal nerves. Studies have shown that CS returns to preoperative levels within 6–12 months following CXL.[29–31] In our study, the CS reached preoperative values in the HMW-HA group in the 3rd month postsurgery, when it still remained impaired in the control and LMW-HA groups. Despite the SNP parameters not fully returning to baseline at the postoperative 3rd month in the HMW-HA group, the recovery of CS suggests that clinical function and morphology may not always be directly correlated. Furthermore, despite corneal nerves not fully recovering in either the control or LMW-HA group at the postoperative 6th month, the CS in the LMW-HA group had already returned to baseline while that of the control group still remained impaired. This result suggests that the long-term use of artificial tears containing HA may contribute to functional improvement by supporting the ocular surface.

The loss of corneal sensation can lead to blinking abnormalities and a decrease in essential tear secretion.[32] Since CXL has the potential to impact corneal sensation, its effects on the ocular surface have been extensively investigated in numerous studies.[24 33–35] Akgöz *et al* reported no evidence of a decline in tear volume or exacerbation of dry eye symptoms following CXL.[24] Notably, it should be mentioned that they performed the CXL procedure using the epi-on technique. Contrarily, Wang *et al* found significant improvement in OSDI and NI-TBUT values at the postoperative 12-month follow-up for both epi-off and epi-on CXL procedures.[35] In our study, the control group had higher OSDI compared with the HA groups, while NI-TBUT was lower. However, NITBUT levels of all groups remained stable during follow-ups. As mentioned by Mazzotta *et al*, the corneal flattening effect of CXL and the formation of a healthier corneal epithelium could potentially explain the stabilisation of TBUT levels observed post-CXL.[5] While it can be stated that CXL does not cause tear film instability, the use of artificial tears, particularly those with HA, seems to be beneficial in promoting ocular surface health and symptoms.

The primary objective of CXL is to stabilise the refractive and biomechanical properties of the cornea, aiming to improve vision. In our study, UCVA, CDVA, SE and keratometric parameters remained stable after 12 months following CXL in all groups.

One of the limitations of this study is that the LMW-HA formulation used also contained trehalose since a pure LMW-HA preparation is not available in our country. Considering the bioprotective effects of trehalose on the ocular surface, it remains unclear whether the observed corneal recovery effect can be only attributed to LMW-HA. Although the study was not conducted as a double-blind trial, objective measures were used to help mitigate this risk.

In conclusion, this study demonstrated the application of HMW-HA eye drops accelerates corneal nerve regeneration and the recovery of CS after CXL. Furthermore, this treatment is more effective in improving ocular surface parameters and clinical symptoms. Clinically, HMW-HA may be preferred over LMW-HA in cases where corneal nerve regeneration is crucial, such as after ocular surgery or in patients with underlying inflammatory conditions like dry eye disease. Future studies should explore the benefits of HMW-HA in corneal nerve regeneration in other conditions, as well as the cellular and molecular mechanisms by which HMW-HA contributes to it.

Contributors Conceptualisation: GÖ, SAT, ET. Methodology: GÖ, SAT, ET. Validation: GÖ, SAT. Investigation: GÖ, SAT. Formal analysis: GÖ, SAT. Writing – original draft: GÖ, SAT. Writing – review and editing: GÖ, SAT, ET. Data curation: SAT, GÖ, ET. Visualisation: GÖ, SAT. Supervision: SAT, ET. Project administration: GÖ, SAT, ET. Resources: GÖ, SAT. GÖ is the guarantor of this study and takes full responsibility for the integrity of the data and the accuracy of the analysis.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. This study was conducted in accordance with the ethical standards defined by the Declaration of Helsinki and Good Clinical Practice after receiving approval from the institutional review board at Marmara University of İstanbul (Protocol No: 09.2021.86). Prior to enrolment and randomisation, informed consent was obtained from all study subjects.

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REFERENCES

1 Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003;135:620–7.
2 Raiskup F, Theuring A, Pillunat LE, *et al*. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: ten-year results. *J Cataract Refract Surg* 2015;41:41–6.
3 Caporossi A, Mazzotta C, Baiocchi S, *et al*. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol* 2010;149:585–93.
4 Wittig-Silva C, Chan E, Islam FMA, *et al*. A randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus: three-year results. *Ophthalmology* 2014;121:812–21.
5 Mazzotta C, Traversi C, Baiocchi S, *et al*. Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. *Am J Ophthalmol* 2008;146:527–33.
6 Knappe S, Stachs O, Zhivov A, *et al*. Results of confocal microscopy examinations after collagen cross-linking with riboflavin and UVA light in patients with progressive keratoconus. *Ophthalmologica* 2011;225:95–104.
7 Mazzotta C, Hafezi F, Kymionis G, *et al*. In Vivo Confocal Microscopy after Corneal Collagen Crosslinking. *Ocul Surf* 2015;13:298–314.
8 Jordan C, Patel DV, Abeysekera N, *et al*. In vivo confocal microscopy analyses of corneal microstructural changes in a prospective study of collagen cross-linking in keratoconus. *Ophthalmology* 2014;121:469–74.
9 Kymionis GD, Diakonis VF, Kalyvianaki M, *et al*. One-year follow-up of corneal confocal microscopy after corneal cross-linking in patients with post laser in situ keratosmileusis ectasia and keratoconus. *Am J Ophthalmol* 2009;147:774–8, .
10 Labetoulle M, Baudouin C, Calonge M, *et al*. Role of corneal nerves in ocular surface homeostasis and disease. *Acta Ophthalmol* 2019;97:137–45.
11 Müller LJ, Marfurt CF, Kruse F, *et al*. Corneal nerves: structure, contents and function. *Exp Eye Res* 2003;76:521–42.
12 Litwiniuk M, Krejner A, Speyrer MS, *et al*. Hyaluronic Acid in Inflammation and Tissue Regeneration. *Wounds* 2016;28:78–88.
13 Preston M, Sherman LS. Neural stem cell niches: roles for the hyaluronan-based extracellular matrix. *Front Biosci (Schol Ed)* 2011;3:1165–79.

14 Kojima T, Nagata T, Kudo H, *et al*. The Effects of High Molecular Weight Hyaluronic Acid Eye Drop Application in Environmental Dry Eye Stress Model Mice. *Int J Mol Sci* 2020;21:3516.
15 Beck R, Stachs O, Koschmieder A, *et al*. Hyaluronic Acid as an Alternative to Autologous Human Serum Eye Drops: Initial Clinical Results with High-Molecular-Weight Hyaluronic Acid Eye Drops. *Case Rep Ophthalmol* 2019;10:244–55.
16 Di G, Qi X, Zhao X, *et al*. Efficacy of Sodium Hyaluronate in Murine Diabetic Ocular Surface Diseases. *Cornea* 2017;36:1133–8.
17 van Setten G-B, Stachs O, Dupas B, *et al*. High Molecular Weight Hyaluronan Promotes Corneal Nerve Growth in Severe Dry Eyes. *J Clin Med* 2020;9:3799.
18 Spadea L, Marrazzo G. Improvement of symptoms post-epi-off CXL by an ophthalmic solution containing 0.05% cyanocobalamine, 0.5% taurine and 0.5% long-chained hyaluronic acid. *Invest Ophthalmol Vis Sci* 2015;56:3012–12.
19 Ondes Yilmaz F, Kepez Yildiz B, Tunc U, *et al*. Comparison of topical omega-3 fatty acids with topical sodium hyaluronate after corneal crosslinking : Short term results. *Ocul Immunol Inflamm* 2022;30:959–65.
20 Ozek D, Kemer OE. Effect of the bioprotectant agent trehalose on corneal epithelial healing after corneal cross-linking for keratoconus. *Arq Bras Oftalmol* 2018;81:505–9.
21 Sabur H, Acar M. Dexpanthenol/sodium hyaluronate eye drops for corneal epithelial healing following corneal cross-linking in patients with keratoconus. *Int Ophthalmol* 2023;43:3461–9.
22 Schulz KF, Altman DG, Moher D, *et al*. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *PLoS Med* 2010;7:e1000251.
23 Irkec MT. Reliability and validity of turkish translation of the ocular surface disease index (OSDI) in dry eye syndrome. *Invest Ophthalmol Vis Sci* 2007;48:408–08.
24 Akgöz H, Fındık H, Aslan MG. Evaluation of tear parameters and meibomian gland morphology in keratoconus patients after epithelial-on corneal cross-linking. *Eur J Ophthalmol* 2022;2022:11206721221118740.
25 Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;22:640–50.
26 van Setten G-B, Baudouin C, Horwath-Winter J, *et al*. The HYLAN M Study: Efficacy of 0.15% High Molecular Weight Hyaluronan Fluid in the Treatment of Severe Dry Eye Disease in a Multicenter Randomized Trial. *J Clin Med* 2020;9:3536.
27 Wu M, Hill LJ, Downie LE, *et al*. Neuroimmune crosstalk in the cornea: The role of immune cells in corneal nerve maintenance during homeostasis and inflammation. *Prog Retin Eye Res* 2022;91:101105.
28 Aragona P, Simmons PA, Wang H, *et al*. Physicochemical Properties of Hyaluronic Acid-Based Lubricant Eye Drops. *Transl Vis Sci Technol* 2019;8:2.
29 Wasilewski D, Mello GHR, Moreira H. Impact of collagen crosslinking on corneal sensitivity in keratoconus patients. *Cornea* 2013;32:899–902.
30 Ozgurhan EB, Celik U, Bozkurt E, *et al*. Evaluation of subbasal nerve morphology and corneal sensation after accelerated corneal collagen cross-linking treatment on keratoconus. *Curr Eye Res* 2015;40:484–9.
31 Parissi M, Randjelovic S, Poletti E, *et al*. Corneal Nerve Regeneration After Collagen Cross-Linking Treatment of Keratoconus: A 5-Year Longitudinal Study. *JAMA Ophthalmol* 2016;134:70–8.
32 Al-Aqaba MA, Dhillon VK, Mohammed I, *et al*. Corneal nerves in health and disease. *Prog Retin Eye Res* 2019;73:100762.
33 Recalde JI, Acera A, Rodríguez-Agirretxe I, *et al*. Ocular Surface Disease Parameters After Collagen Cross-Linking for Keratoconus. *Cornea* 2017;36:148–52.
34 Uysal BS, Akcay E, Kilicarslan A, *et al*. Tear function and ocular surface changes following corneal collagen cross-linking treatment in keratoconus patients: 18-month results. *Int Ophthalmol* 2020;40:169–77.
35 Wang X, Bai X, Jin C, *et al*. Analysis of Tear Function Outcomes following Collagen Cross-Linking Treatment in Ectatic Corneas. *J Ophthalmol* 2022;2022:1910607.