ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Comparison of Corneal Endothelial Parameters and Intraocular Pressure Alterations After Uneventful Cataract Surgery with 1.8% and 3% Sodium Hyaluronate

%1.8 ve %3 Sodyum Hiyalüronat ile Komplikasyonsuz Katarakt Cerrahisi Sonrası Kornea Endotel Parametreleri ve Göz İçi Basıncı Değişikliklerinin Karşılaştırılması

Emre Aydin[1], Hasan Akgoz[2], Mehmet Gokhan Aslan[3]

[1]Department of Ophthalmology, Faculty of Medicine, Samsun University, Samsun; [2]Department of Ophthalmology, Erzurum City Hospital, Erzurum; [3]Department of Ophthalmology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Türkiye

ABSTRACT

Aim: *To assess the effectiveness and safety of two recently developed sodium hyaluronate (Na-Hy) items, differentiated by concentrations of 1.8% and 3%.*

Material and Method: *Fifty-nine uneventful phacoemulsification cataract surgeries were retrospectively evaluated. Patients were separated into two groups according to the ocular viscoelastic device (OVD) used in the capsulorhexis and phacoemulsification steps of the surgery. There were 30 patients in the 3% Na-Hy group and 29 patients in the 1.8% Na-Hy group. Patients were evaluated regarding endothelial changes by specular microscopy and postoperative intraocular pressure (IOP) alterations. Patients with systemic diseases, intraoperative complications, or mature cataracts were excluded.*

Results: *The two groups had no significant difference in demographic characteristics. Mean phaco time was 10.8±3.58 seconds in the 3% Na-Hy group and 10.55±3.85 seconds in the 1.8% Na-Hy group. The preoperative, postoperative 1st, and 3rd months endothelial cell density (ECD) measurements were 2525.8±219.7, 2304.7±197.6 and 2230.6±208.8 cells/mm^2 in the 3% Na-Hy group, and 2549.7±222.4, 2256.6±198.4 and 2166.3±201.5 cells/mm^2 in the 1.8% Na-Hy group, respectively. The preoperative, postoperative 1st day and 1st week IOP measurements were 17.1±2.56, 20.5±3.82 and 15.76±2.19 in 3% Na-Hy group and 15.96±2.56, 18.1±3.35 and 14.93±2.15 in 1.8% Na-Hy group, respectively. The reduction in endothelial cell density was notably greater in the group treated with 1.8% Na-Hy. At the same time, no significant differences were observed among the groups regarding changes in postoperative intraocular pressure (IOP).*

Conclusion: *Both Na-Hy products seem safe and effective for routine cataract cases. The 3% Na-Hy showed a better performance for corneal endothelium protection while causing minimal IOP elevation.*

Key words: *Hyotek; ocular viscoelastic device; phacoemulsification; sodium-hyaluronate*

ÖZET

Amaç: *Yeni geliştirilen, %1,8 ve %3 konsantrasyonlara farklılaştırılmış iki sodyum hiyalüronat (Na-Hy) ürününün etkinliğini ve güvenliğini değerlendirmek.*

Materyal ve Metot: *Elli dokuz komplikasyonsuz fakoemülsifikasyon katarakt ameliyatı retrospektif olarak değerlendirildi. Hastalar ameliyatın kapsüloreksis ve fakoemülsifikasyon aşamalarında kullanılan oküler viskoelastik maddeye (OVD) göre iki gruba ayrıldı. %3 Na-Hy grubunda 30, %1,8 Na-Hy grubunda ise 29 hasta vardı. Hastalar speküler mikroskopi ile endotel değişiklikleri ve ameliyat sonrası göz içi basıncı (GİB) değişiklikleri açısından değerlendirildi. Sistemik hastalıkları, intraoperatif komplikasyonları veya matür kataraktı olan hastalar çalışma dışı bırakıldı.*

Bulgular: *İki grup arasında demografik özellikler açısından anlamlı bir fark yoktu. Ortalama fako süresi %3 Na-Hy grubunda 10,8±3,58 saniye, %1,8 Na-Hy grubunda ise 10,55±3,85 saniye idi. Preoperatif, postoperatif 1. ve 3. ay endotel hücre yoğunluğu ölçümleri sırasıyla %3 Na-Hy grubunda 2525,8±219,7, 2304,7±197,6 ve 2230,6±208,8 hücre/ mm2 idi ve 1,8 Na-Hy grubunda ise 2549,7±222,4, 2256,6±198,4 ve 2166,3±201,5 hücre/ mm2 idi. Ameliyat öncesi, ameliyat sonrası 1. gün ve 1. hafta GİB ölçümleri sırasıyla %3 Na-Hy grubunda 17,1±2,56, 20,5±3,82 ve 15,76±2,19, %1,8 Na-Hy grubunda ise 15,96±2,56, 18,1±3,35 ve 14,93±2,15 idi. Endotel hücre yoğunluğundaki azalma, %1,8 Na-Hy ile tedavi edilen grupta belirgin şekilde daha fazlaydı, ancak postoperatif göz içi basıncındaki (GİB) değişiklikler açısından gruplar arasında anlamlı bir fark gözlenmedi.*

Sonuç: *Her iki Na-Hy ürünü de rutin katarakt vakalarında güvenli ve etkili görünmektedir. %3 Na-Hy, minimal GİB yükselmesine neden olurken kornea endotelini koruma konusunda daha iyi bir performans gösterdi.*

Anahtar kelimeler: *Hyotek; oküler viskoelastik madde; fakoemülsifikasyon; sodyum-hyalüronat*

İletişim/Contact: *Hasan Akgoz, Erzurum City Hospital, Erzurum, Türkiye* • ***Tel:*** *0506 672 96 35* • ***E-mail:*** *dr.hasanakgoz@gmail.com* • ***Geliş/Received:*** *23.11.2023* • ***Kabul/Accepted:*** *22.01.2024*

ORCID: *Emre Aydın: 0000-0002-1895-8538* • *Hasan Akgöz: 0000-0003-1115-8359* • *Mehmet Gökhan Aslan: 0000-0002-3250-1606*

Introduction

Cataract surgery is one of the most performed surgical interventions in the modern world. Advances in phacoemulsification energy delivery technologies and disposable products in the last three decades have provided excellent surgical results. However, new research is still on track to solve the issues for challenging eyes[1].

The loss of endothelial cells during cataract surgery leads to prolonged corneal edema and, in severe cases, may cause corneal decompensation and decreased final visual acuity[2]. Since lens removal surgery has gained a refractive surgical identity, the protection of corneal endothelium has become even more important[3]. Ocular viscoelastic devices (OVDs) are widely used to facilitate surgical procedures and reduce the risk of secondary damage to delicate intraocular tissues. They maintain anterior chamber space and stability to prevent capsular rupture and provide clarity to avoid complications[4]. These properties vary with the physical, chemical, and rheological properties of OVDs[5]. However, a longer retention time is a significant drawback of OVDs for cataract surgery. That may cause IOP elevations within the postoperative 24 hours, which may negatively impact glaucoma patients[6,7].

The OVDs are available in different concentrations and materials for particular needs. The Na-Hyaluronate (Na-Hy) is cohesive in lower concentrations but becomes more dispersive as the concentration increases. Kretz et al. reported that 3% Na-Hy covered a significantly higher rate of endothelium than 1.4% Na-Hy in porcine eyes[8]. However, Holzer et al. detected higher IOP peaks at the postoperative 4th hour with higher concentrations of OVDs[9]. Even though the OVDs are classified by their Na-Hy percentage, numerous other parameters, such as viscosity, density, and molecular weight, define their properties. Hyotek (Teknomek, Istanbul, Türkiye) is a new OVD brand available in several concentrations of Na-Hy at the same molecular weight. Therefore, they provide optimum solutions for any challenging condition in cataract surgery.

This study evaluated the corneal parameters and early postoperative IOP alterations after cataract surgery with Hyotek 3% and 1.8% Na-Hyaluronate OVDs utilized in capsulorhexis and phacoemulsification stages. Hence, the aim was to evaluate their safety and efficiency.

Materials and Methods

Ethical Approval

Before beginning the research, the Institutional Ethics Committee (E-40465587-050.01.04-131) approved it, and all researchers agreed to follow the principles of the Helsinki Declaration. The study was explained to the patients verbally, and a signed agreement was obtained from all participants.

Subject Recruitment

This retrospective observational study included 59 cataract patients aged between 50 and 80 who underwent uncomplicated surgery by the same surgeon with an Oertli Faros phacoemulsification device in a tertiary eye clinic between October 2020 and March 2021. The patients were randomly assigned into two groups: the study group with 3% Na-Hyaluronate (Hyotek, Teknomek, Istanbul, Türkiye) during the capsulorhexis and phacoemulsification stages of the surgery, and the control group with 1.8% Na-Hyaluronate (Hyotek, Teknomek, Istanbul, Türkiye) in the same stages. The technical specifications of both OVDs are shown in Table 1. The 1.4% Na-Hy injected foldable intraocular lens in all groups.

The corrected distance visual acuity (CDVA), anterior segment biomicroscopy, and corneal endothelial parameters were recorded preoperatively and 1 and 3 months after surgery. The IOP measurements were also recorded one day after the procedure. Endothelial cell density (ECD), central corneal thickness (CCT), average cell area (AVG), and cell volume (CV) were measured by noncontact specular microscopy (Tomey EM-4000, Nagoya, Japan) at each visit[10].

The corneal edema was evaluated on 1st postoperative day with the biomicroscope. It was noted as four

Table 1. *Technical specifications of the 1.8% and 3% Na-hyaluronate (Hyotek) OVDs*

Sodium Hyaluronate	Hyotek 1.8%	Hyotek 3 %
Molecular Weight	2.3-3.2 MDa	2.3-3.2 MDa
pH*	6.8-7.6	6.8-7.6
Density*	0.998-1.008 g/ml	1.001-1.020 g/ml
Viscosity*	150,000-300,000mPa.s	400,000-900,000mPa.s
Tolerance*	NA	+70,000 mPa.s
Osmolality*	200-400mOsm/kg	200-400mOsm/kg
Endotoxicity*	<0.2EU/ml	<0.2EU/ml
Refractive Index*	1.337-1.338	1.337-1.338

*After sterilization
MDa: Megadaltons; **g:** Gram; **ml:** Milliliter; **mPa.s:** Milli pascal second; **mOsm:** Milliosmole; **kg:** Kilogram; **EU:** Endotoxin units.

grades: Grade 0 (no corneal haze), Grade 1 (iris details visible), Grade 2 (pupil margin visible but iris details not visible), Grade 3 (pupil margin not visible), and Grade 4 (cornea opaque)[11].

Patients with a history of autoimmune disorder (e.g., rheumatoid arthritis, inflammatory bowel disease), corneal disorder, mature and/or Morgagnian cataract, and ocular trauma/surgery were excluded. Besides, complicated surgeries such as posterior capsule rupture, vitreous loss, postoperative inflammatory pupillary membrane, cystoid macular edema, drug allergy and/or toxicity, and patients with any missing data were also excluded.

Surgical Procedure

Local anesthesia was applied with a peribulbar injection of 4 mL 2% lidocaine. The conjunctiva was washed with 5% povidone-iodine for 3 minutes after sterile draping. Two side port incisions were created with a 19G MVR blade. Trypan blue was used to improve the visibility of the capsule. After filling the anterior chamber with 3% (Group 1) and 1.8% (Group 2) sodium hyaluronate (Hyotek, Teknomek, Istanbul, Türkiye) viscoelastic, a self-closing transparent corneal incision was created using a 2.2 mm corneal knife. 5–5.5 mm continuous circular capsulorhexis, hydro dissection, and hydro delineation were performed. In all cases, the cataractous lens was removed with the Oertli Faros phacoemulsification device using the quick chop technique with the same parameters. The cortex remnants were removed with bimanual irrigation/aspiration (I/A). The capsular bag was filled with 1.4% Na-Hy (Hyotek, Teknomek, Istanbul, Türkiye). A foldable hydrophobic intraocular lens was placed inside the capsular bag. The OVD was removed with bimanual I/A, and After administering moxifloxacin hydrochloride 0.5% (Vigamox, Alcon Labs, Fort Worth, TX) to the anterior chamber, corneal incisions were hydrated. All patients were started on antibiotics and steroid drops five times a day in the postoperative period and were given tapering doses for one month.

Statistical Analysis

Statistical analysis was conducted using IBM Statistical Package for Social Sciences (SPSS) program version 23.0 for Windows (IBM, Inc., Chicago, IL). The distribution of variables was examined through analytical methods. Descriptive statistics for normally distributed variables were presented as mean ± standard deviation. Categorical variables were analyzed using the chi-square test. Repeated-measures ANOVA was employed to compare normal values at different time points, with Bonferroni correction applied for multiple comparisons. Group differences for normally distributed variables were assessed using the independent samples t-test. All analyses were conducted with a 95% confidence interval. Statistical significance was set at $P < 0.05$.

Results

The mean age of the 59 patients included in this study was 70.23±7.10. Thirty patients were included in the 3% Na-Hy group and 29 in the 1.8% Na-Hy group. No notable distinctions were observed among the groups concerning age, gender, laterality, and phaco time. The demographic distribution of the patients is shown in Table 2.

There was no significant difference between the patients' baseline visual acuity, intraocular pressure, and corneal parameters ($p>0.05$). There were five patients with weak pupil dilatation in both groups. (16.7% and 17.2%, $p=0.953$). Significant alterations were detected in visual acuity, ECD, AVG, and CCT values in both groups at 1st and 3rd months compared to baseline (Table 3). However, CCT alterations

Table 2. *Demographic data of the patients*

	3% Na-Hyaluronate Group			1.8% Na-Hyaluronate Group			
	Mean	**SD**		**Mean**	**SD**		**p**
Age (years)	69.80	8.16		70.68	5.92		0.635[a]
Phaco Time (second)	10.80	3.58		10.55	3.85		0.799[a]
	N	Right	Left	N	Right	Left	p
Male	15	8	7	12	7	5	0.506[b]
Female	15	9	6	17	10	7	0.604[b]
Total	30	17	13	29	17	12	0.879[b]

Data are presented as mean ± standard deviation (SD).
[a]Independent sample test; [b]Chi-square test.

Table 3. *Alterations of the visual and corneal parameters in time*

	3% Na-Hyaluronate Group				1.8% Na-Hyaluronate Group			
	Pre-op	**1st month**	**3rd Month**	**p**	**Pre-op**	**1st month**	**3rd month**	**p**
CDVA (Snellen)	0.15±0.11	0.89±0.12	0.93±0.09	<0.001	0.14±0.10	0.91±0.09	0.94±0.07	<0.001
ECD (cell/mm²)	2525.8±219.7	2304.7±197.6	2230.6±208.6	<0.001	2549.7±222.4	2256.6±198.4	2166.3±201.5	<0.001
AVG	414.3±37.0	432.6±35.8	434.9±36.2	<0.001	396.8±34.1	415.3±35.1	420.7±35.6	<0.001
CV	37.0±4.2	41.5±4.4	41.8±4.8	<0.001	36.9±5.0	42.0±4.5	43.2±4.3	<0.001
CCT (µm)	533.6±39.4	558.8±40.0	548.4±38.3	<0.001	527.5±31.2	552.1±32.6	543.2±32.0	<0.001

Data are presented as mean ± standard deviation.
CDVA: Corrected distance visual acuity; **ECD:** Endothelial cell density; **AVG:** Avarage; **CV:** Cell volume; **CCT:** Central corneal thickness; **µm:** Micrometer; **mm²:** Square millimeter.
Independent Samples t-test.

Table 4. *Comparison of the alterations of both groups*

	Δ (1st month-Preop)			Δ (3rd month-Preop)			Δ (3rd month-1sth month)		
	3% Na-Hyaluronate	**1.8% Na-Hyaluronate**	**p**	**3% Na-Hyaluronate**	**1.8% Na-Hyaluronate**	**p**	**3% Na-Hyaluronate**	**1.8% Na-Hyaluronate**	**p**
CDVA (Snellen)	0.73±0.11	0.77±0.12	0.271	0.77±0.11	0.80±0.11	0.412	0.04±0.09	0.03±0.06	0.680
ECD (cell/mm²)	-224.4±56.3	-293.1±83.8	<0.001	-298.2±87.5	-383.4±105.9	<0.001	-74.0±64.6	-90.2±68.4	0.434
AVG	18.22±6.25	18.48±8.31	0.897	20.53±13.21	23.93±8.99	0.255	2.30±9.69	5.44±4.04	0.111
CV	4.50±0.97	5.06±1.30	0.062	4.86±1.56	6.31±1.79	0.002	0.36±1.27	1.24±1.02	0.005
CCT (µm)	25.13±6.65	24.58±7.26	0.764	14.76±5.70	15.68±7.15	0.585	-10.36±6.35	-8.89±7.02	0.403

Data are presented as mean ± standard deviation.
CDVA: Corrected distance visual acuity, **ECD:** Endothelial cell density, **AVG:** Avarage, **CV:** Cell volume, **CCT:** Central corneal thickness, **µm:** Micrometer, **mm²:** Square millimeter, **Δ:** Difference. Independent samples t-testp<0.05 significant values were shown in bold.

were insignificant in both groups in the 3rd month compared to preoperative measurements. (p=0.413, p=0.382, respectively)

The groups were compared in terms of ECD alterations over time, and significantly less endothelial loss was observed in the 3% Na-Hy group compared to the 1.8% Na-Hy group in both the 1st and 3rd months. Besides, the CV alteration was significantly lower in the 3% Na-Hy group between 1st-3rd months and preoperative-3rd months (Table 4).

The preoperative, postoperative 1st day, and 1st week IOP measurements were 17.1±2.56, 20.5±3.82 and 15.76±2.19 mm-Hg in 3% Na-Hy group and 15.96±2.56, 18.1±3.35 and 14.93±2.15 mm-Hg in 1.8% Na-Hy group, respectively. In both groups, the IOP value increased significantly on the 1st day. (p=0.011, p=0.043, respectively). However, there was no significant difference between the 1st week and preoperative measurements (Fig. 1).

The patients were also evaluated for corneal edema on the postoperative 1st day. No notable distinction was observed between the two groups concerning corneal edema. However, 3(+) edema was observed in 4 patients in the 1.8% Na-Hy group. In contrast, 3 (+) edema was not detected in any patient operated with 3% Na-Hy (Fig. 2).

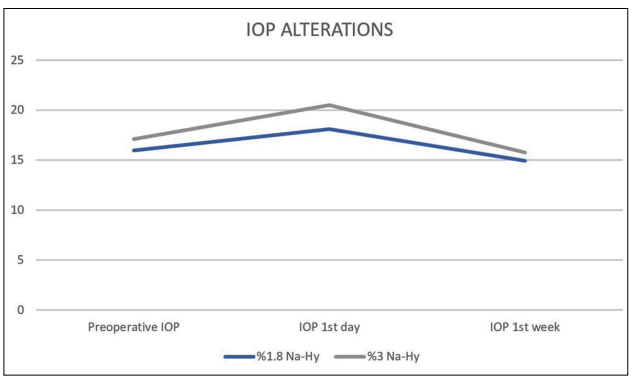

Figure 1. *Comparison of intraocular pressure change between groups.*

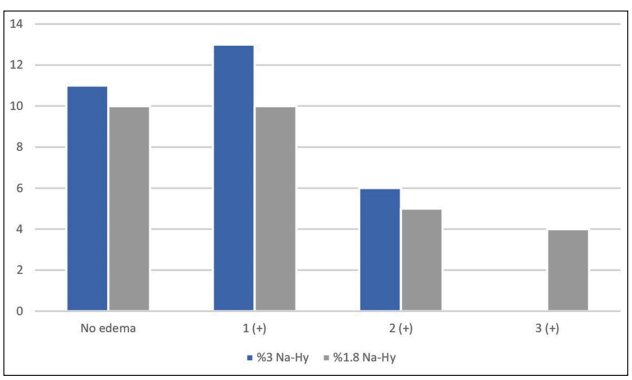

Figure 2. *Comparison of corneal edema change between groups.*

Discussion

This study evaluated the corneal endothelial and IOP alterations after uncomplicated cataract surgeries by the same surgeon (MGA) using 3% NaHy and 1.8% NaHy in capsulorhexis and phacoemulsification stages. There was no difference between the groups regarding baseline characteristics, and the same standard technique was applied. The 3% Na-Hy showed slightly better performance regarding endothelial protection, while there was no statistical difference in IOP alterations.

Cohesive viscoelastic creates a wider anterior chamber to manipulate surgical instruments and protect the corneal endothelium against iatrogenic trauma. Ben-Eliahu et al. compared various concentrations of Na-Hy. They found that all concentrations were significantly superior to irrigation to decrease endothelial oxidative stress[12]. Both 3% Na-Hy and 1.8% Na-Hy used in our study are cohesive viscoelastic. Even though the molecular weights of both concentrations were similar, the viscosity of 3% Na Hyaluronate was higher. Higher viscosity cohesive OVDs can maintain spaces and pressure the eye quite well. However, they may occasionally leave the anterior chamber too quickly in prolonged or complicated surgeries, so they afford less corneal endothelial protection in these circumstances[13]. The endothelial loss was significantly less in this study's 3% Na-Hy group. Therefore, despite the higher viscosity, 3% Na-Hy showed a sufficient performance to avoid unintentional outflow from the anterior chamber, or the higher viscosity Hyotek protects the endothelium better by causing a more expansive interior space.

Researchers have not agreed on the effects of various OVDs on endothelial cell numbers. Holzer et al. reported that Healon 5 (2%.3 Na-Hy) causes less cell loss than other Na-Hys in lower concentrations during phacoemulsification[9]. Goles et al. suggested that removing dispersive OVDs caused slightly higher postoperative endothelial cell loss than irrigation[12]. The mean endothelial cell loss was 16% with dispersive OVDs after uneventful routine phacoemulsification surgery[14]. The ECD loss in either group was assessed in this study via cell density calculation on a specular microscope. It was 11.6% in the 3% Na-Hy group and 15% in 1.8%. Na-Hy group in the 3rd postoperative month. These results suggest that high-viscosity cohesive OVDs have a similar safety index to dispersive OVDs in phacoemulsification surgery. On the other hand, this comparison would be more valuable when evaluated in complex case scenarios such as mature and Morgagnian cataracts.

The IOP alterations after cataract surgery occur on the first postoperative day and usually disappear within 3 days[15]. In particular, insufficient OVD cleaning causes a mechanical blockage at the iridocorneal angle, disrupting aqueous humor flow[16]. Apart from the classical symptoms, increased IOP may even lead to retinal artery occlusion and anterior ischemic optic neuropathy[17]. Besides, that may cause damage to the optic disc in patients with glaucoma, resulting in worsening of the visual field loss[18]. The Na-Hy-induced IOP elevation is due to its high molecular weight and viscosity[19]. While the molecular weights of the two OVDs used in our study are the same, the viscosity of the 3% Na-Hy is higher. This may explain the relatively higher increase in IOP. However, at the end of 24 hours, IOP was at normal levels in both groups, and the IOP did not exceed 30 mm-Hg in any patient. The OVDs in both concentrations were quickly removed from the anterior chamber, and the lack of significant IOP change between them can be explained by the meticulous cleaning after IOL implantation before hydrating stroma.

The migration and enlargement of central corneal endothelial cells exhibit their maximum cell density and size response, albeit delayed until three months post-surgery[20]. The Oxford Cataract Treatment and Evaluation Team recommends performing an endothelial cell count at least 90 days postoperatively, aligning with our study, to ensure stability in cell reorganization and loss[21]. Analyzing endothelial cell size and shape is a more sensitive indicator than cell count[22]. The variation coefficient (CV), a non-dimensional index ensuring a quantitative measurement of cell size variation (polymegethism), becomes crucial[23]. Matsuda et al. observed a rapid decline in central endothelial cell density and disruption of normal morphology after intracapsular cataract extraction without implant in the first month. They reported a gradual recovery of hexagonal cell frequency over 1 to 6 months postoperatively, ultimately restoring cellular morphology to normal[24]. Another study found that the size CV normalized due to the reorganization of endothelial cells one month after surgery[25]. Another study found that the size of CV normalized one month after surgery due to endothelial cell reorganization. In our study, the number of CVs in both groups changed and increased in the postoperative 1st and 3rd months compared to preoperative values. Still, this increase did not reach statistical significance. The difference in preoperative and postoperative 1st-month CV counts was similar in both groups. The increase in the number of CVs

from the 1st to the 3rd month postoperatively was significantly higher in the 1.8% Na-Hy group. That can be explained by the fact that the morphology of the cells improved quicker compared to the 3% Na-Hy group to tolerate the decrease in the ECD count since the decrease in the ECD in the postoperative 1st month was statistically higher with 1.8% Na-Hy.

Isoosmotic aqueous humor replaces OVDs after surgery, and the osmotic agent's effect rapidly diminishes. Kiss et al. showed that CCT returned to preoperative values in the postoperative 3rd month[26]. In our study, there was an increase in CCT in both groups in the postoperative 1st month and 3rd month compared to preoperative values. However, the CCT decreased in both groups in the 3rd month compared to the 1st month. A possible mechanism might be prolonged inflammation that causes corneal changes rather than the swelling of the cornea as a result of the endothelial disruption. However, long-term studies with specific measurements of each corneal layer are needed to demonstrate particular alterations. The corneal edema was significantly lower in the 3% Na-Hy group. Two mechanisms can explain that. Firstly, the low-viscosity Na-Hy may create less space during surgery, which increases the risk of instruments and/or phacoemulsification energy damaging the corneal endothelium. Besides, 3% OVDs with higher binding affinity to the corneal endothelium may cover the endothelium during phacoemulsification and irrigation/aspiration[7].

Thus, the 3% Na-Hy forms a thicker layer on the endothelium, or its binding affinity might be stronger. As the viscosity of OVDs increases, they contain a higher concentration of hyaluronan or longer chains[27]. Hence, it can be concluded that NaHy, which has a higher viscosity, can bind more to hyaluronate binding sites in the endothelium and protect the cornea better since its hyaluronan concentration is higher.

Our study had certain limitations. Firstly, the number of cases was limited, and only uneventful procedures were included. That may prevent reaching a generalized conclusion. Besides, excluding high-grade mature cataracts might prevent the evaluation of the full performance of OVDs in terms of endothelial protection. Therefore, studies assessing the performance of these OVDs in larger sample-sized groups with a wide variety of cataract populations in long-term studies are necessary. Moreover, as this study proved their safety and efficacy, these OVDs can be compared with brands containing similar molecules and concentrations.

Conclusion

As a result, these newly released OVDs containing 1.8% and 3% Na-Hy concentrations were found to be safe and effective for cataract surgery. As anticipated, the OVD containing 3% Na-Hy demonstrated greater efficacy in safeguarding endothelial function. However, no notable distinctions were detected among the groups regarding changes in IOP. Choosing 3% Na Hy in cataract patients with previous congenital and/or acquired corneal disorders may positively impact the restoration of vision after surgery.

Conflict of Interests

All authors have no conflict of interest.

Statement of Ethics

All researchers agreed to apply the tenets of the Declaration of Helsinki.

The institutional review board of X University (E-40465587-050.01.04-131) approved the study, and all participants signed a written informed consent form.

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