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Corneal Endothelial Cell Loss After Phacoemulsification in Post-Penetrating Keratoplasty Eyes with Domestic and Imported Donor Cornea: Cross-Sectional Research

Yerli ve İthal Donör Kornealı Penetran Keratoplasti Yapılan Gözlerde Fakoemülsifikasyon Sonrası Kornea Endotel Hücre Kaybı: Kesitsel Araştırma

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ABSTRACT Objective: To compare the changes in endothelial cell density (ECD) in patients who underwent cataract extraction with phacoemulsification after penetrating keratoplasty (PK) with domestic and imported corneas. **Material and Methods:** Patients who had PK with domestic (Group 1) and imported (Group 2) donor corneas underwent phacoemulsification between April 2013 and June 2014 were included in this study. ECD was evaluated with noncontact specular microscopy before and 1st, 3rd, 6th, 12th months after cataract surgery. **Results:** In Group 1, 15 eyes of 15 patients and in Group 2, 13 eyes of 13 patients were evaluated. No significant differences were observed between the groups with respect to age, gender, laterality, indication for PK, nuclear opacity, time interval between PK and cataract surgery (p>0.05). The domestic group had a lower donor age (p=0.049), shorter preservation-to-surgery times and more ECD (p<0.001). The mean corneal ECD was 2910.73±312.16 (2451-2454) cells/mm^2 in Group 1 and 2463.54±236.99 (2043-2876) cells/mm^2 in Group 2 preoperatively (p<0.001). ECD was significantly higher in domestic donor corneas compared to imported donor corneas before PK, before cataract surgery, and at all controls after cataract surgery (p<0.05, for each). The percentage of mean decrease in the number of ECD between postoperative 1st-3rd month, 3rd-6th month and for the 6th-12th month was statistically higher in imported group than in the domestic group (p<0.05, for each). **Conclusion:** Related to the amount of change in ECD after cataract surgery, imported corneas seem as credible as domestic corneas. To reach the exact outcome, more detailed and longlasting studies are necessary.

Keywords: Penetrating keratoplasty; imported cornea; domestic cornea; donor cornea; eyebank

ÖZET Amaç: Yerli ve ithal kornealarla penetran keratoplasti (PK) sonrası fakoemülsifikasyon ile katarakt ekstraksiyonu yapılan hastalarda endotel hücre yoğunluğundaki [endothelial cell density (ECD)] değişiklikleri karşılaştırmak. **Gereç ve Yöntemler:** Bu çalışmaya Nisan 2013-Haziran 2014 tarihleri arasında yerli donör kornea (Grup 1) ve ithal donör kornea (Grup 2) ile PK sonrası fakoemülsifikasyon yöntemi ile katarakt cerrahisi olan hastalar dâhil edildi. ECD, katarakt cerrahisi öncesi ve sonrası 1, 3, 6, 12. aylarda temassız speküler mikroskopi ile değerlendirildi. **Bulgular:** Grup 1'de 15 hastanın 15 gözü ve Grup 2'de 13 hastanın 13 gözü değerlendirildi. Gruplar arasında yaş, cinsiyet, lateralite, PK endikasyonu, nükleer opasite ve PK ile katarakt cerrahisi arasındaki süre açısından anlamlı fark gözlenmedi (p>0,05). Yerli grubun donör yaşı daha düşük (p=0,049), ameliyata kadar geçen süre daha kısa ve daha fazla ECD (p<0,001) vardı. Ameliyat öncesi ortalama kornea ECD'si Grup 1'de 2910,73±312,16 (2451-2454) hücre/mm^2 ve Grup 2'de 2463,54±236,99 (2043-2876) hücre/mm^2 idi (p<0,001). ECD, PK öncesi, katarakt ameliyatı öncesi ve katarakt ameliyatı sonrası tüm kontrollerde ithal donör kornealara kıyasla yerli donör kornealarda önemli ölçüde daha yüksekti (p<0,05). ECD ortalama azalma yüzdesi, ameliyat sonrası 1-3. ay, 3-6. ay ve 6-12. ay arasında yerli gruba kıyasla ithal grupta grupta istatistiksel olarak daha fazla idi (her biri için p<0,05). **Sonuç:** Katarakt cerrahisi sonrası ECD'deki değişim miktarı ile ilgili olarak ithal kornealar yerli kornealar kadar güvenilir görünmektedir. Kesin sonuca ulaşmak için daha detaylı ve uzun süreli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Penetran keratoplasti; ithal kornea; yerli kornea; donör kornea; göz bankacılığı

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The most effective tissue transplant is penetrating keratoplasty (PK).[1] The factors affecting graft survival, which are positively correlated to the success of PK, are transplantation of the donor cornea with sufficient number of endothelial cell density (ECD) in the preoperative period and preservation of the ECD against aging, surgical operations, and complications acquired in the postoperative period.[2-6] Among the surgical procedures, cataract surgery is the most common secondary surgery in PK eyes.[7-10] Cataract after PK can develop due to aging, lens damage in the course of PK procedure and extended use of corticosteroidal agents.

A previous study from our clinic examined the graft survival in PK surgery with domestic and imported donor corneas and reported that imported and donor corneas did not differ in terms of graft survival, although domestic donor corneas had a lower donor age ($p=0.012$), shorter donor death-to-preservation time, and preservation-to-surgery time ($p<0.001$), and higher ECD ($p<0.001$).[5] Due to increased international cornea sharing in recent years, it is important to know the ECD change after cataract surgeries in eyes which have undergone PK with imported corneas. To compare the changes in ECD and morphology in patients who underwent cataract extraction with phacoemulsification after PK with domestic and imported corneas were aimed in this study.

MATERIAL AND METHODS

This study was authorized by the Ankara Training and Research Hospital Ethics Committee and carried out in compliance with the guidelines of the Declaration of Helsinki's 2013 amendment (date: August 18, 2021, no: E-21-640). The medical records of patients who underwent cataract extraction with phacoemulsification after PK with domestic and imported corneas were retrospectively screened. Patients who developed cataract after PK were included in the study. Patients underwent lamellar keratoplasty, re-keratoplasty, cataract surgery simultaneously with corneal transplantation and patients with preoperatively glaucoma, graft rejection after PK, rheumatic disease, uveitis, autoimmune vesiculobullous disorders of the conjunctiva, corneal neovascularization that can trigger the inflammation after cataract surgery and under the age of 18 were excluded.

The cataract was graded using The Lens Opacities Classification System III was used to grade the cataract following a slit lamp examination.[11] Prior to treatment, the patients in every case received information regarding the characteristics of the surgery and any potential side effects.

On the basis of the availability of donor tissue, the patients who underwent cataract extraction with phacoemulsification after PK were divided into two groups: Group 1 included cases where PK was performed with domestic donor corneas (15 eyes of 15 patients); Group 2 included cases where PK was performed with imported donor corneas (13 eyes of 13 patients).

PREPARATION OF DONOR CORNEA

Domestic donor corneas were received from the International Eye Bank of the Ankara Training and Research Hospital and kept in the Eusol-C facility (AL.CHI.MI.A. S.r.l., Viale Austria 14, 35020 Ponte S. Nicolo (PD)-Italy). All imported corneas came from Tissue Bank International, were kept in Optisol-GS (Bausch & Lomb, USA), and approved after the examination of the donor by the eyebank director using a biomicroscope and count ECD (Conan, Eye Bank Kerato Analyzer EKA-10). Corneas with an ECD of 2000 or above were granted approval.

On the day of surgery, all corneas were transferred to a refrigerator in the operating room after being preserved at 4 °C in a refrigerated in the institutional eyebank. They were used after reaching room temperature about an hour before the operation.

SURGICAL TECHNIQUE

Penetrating Keratoplasty

In every case receiving PK, the normal microsurgery procedure was applied. Using a punch trepan, donor cornea that was 0.25 to 0.50 mm bigger than the recipient bed was removed. After marking the corneal center, a vacuum trepan was used to prepare the recipient bed. The graft was stitched with interrupted or continuous sutures, or a mix of these, and the knots were imbedded into the recipient cornea after

the installation of 4 cardinal sutures with a 10-0 nylon. The viscoelastic substance utilized was sodium hyaluronate, 1%. When necessary, anterior vitrectomy and synechiotomy were performed. At the conclusion of the procedure, a balanced salt solution was used to create the anterior chamber, and subconjunctival injections of gentamycin (40 mg/0.5 mL) and dexamethasone (4 mg/1 mL) were given.

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Cataract Surgery with Phacoemulsification

The same surgeon (AB) carried out all operations. After general and sub-Tenon anesthesia with lidocaine hydrochloride 2.0% and bupivacaine hydrochloride 0.5%, cataract surgery was completed. After creating a small conjunctival peritomy at the superior quadrant, the Tenon's layer is focally removed and light bipolar cautery was applied for hemostasis. The crescent blade was used to make a half-scleral depth incision, parallel to the limbus and about 1-3 mm posterior to the surgical limbus and then used to enter the scleral groove at a chosen depth and dissect anteriorly, parallel to the corneoscleral surface and into clear cornea, making a tunnel incision. The anterior chamber was equilibrated with viscoelastic containing 0.5 mL of chondrotin sulfate-sodium hyaluronate (Viscoat, Alcon Laboratories, Inc., Fort Worth, Texas, USA). Hydrodissection was carried out following the creation of a continuous, curvilinear capsulorhexis with a diameter of roughly 5.5 mm. The nucleus was phacoemulsified endocapsularly, and the remaining lens material was aspirated 1% sodium hyaluronate was used to fill the capsular bag. Afoldable tree-piecehydrophobic intraocular lens (AcrySof IOL, Alcon Laboratories, INC, Fort Worth, Texas, USA) wasplaced in the capsular bag. After aspiration of the viscoelastic material, the scleral tunnel is sutured with a single 10-0 nylon with the knot rotated and buried and conjunctiva was closed with 10-0 nylon sutures.

Postsurgical Treatment

Topical 1% prednisolone acetate and topical 0.5% moxifloxacin were applied eight times daily during the postoperative period. Topical prednisolone acetate tapered over one month and topical moxifloxacin tapered over two weeks. Patients who underwent PK due to herpetic keratitis also began oral acyclovir medication (5x400 mg) after surgery in addition to the mentioned topical treatment. The prophylactic dosage was modified in light of the patients' clinical circumstances. The regimen was supplemented as needed with topical lubricants, topical antiglaucoma medications, and topical/systemic cyclosporin-A.

FOLLOW-UP

At the cornea section, all cases were routinely checked at one day, one week, and four weeks following the treatment, as well as at two, three, six, nine, twelve, eighteen months, and 24 months, and then every year after that. The intraocular pressure, ECD, anterior and posterior segment examinations were performed at each examination, and the best corrected visual acuity was assessed using the Snellen chart. The intraocular pressure measured with Goldmann applanation.

Preoperatively ECD and morphology of donor corneas were evaluated by Konan specular microscopy (Eye Bank Kerato Analyzer EKA-10, Konan, Hyogo, Japan). Using a noncontact specular microscope (Konan Specular Microscope X NSP-9900 Hyogo, Japan), the ECD and percentage hexagonality were assessed at 1, 3, 6, and 12 months following surgery. The ECD was obtained from the image of the cornea where the boundaries of the biggest area of cells were clearly visible. The number

of cells within the designated area was counted, and the ECD was calculated using that number. The demographic information about the patients, the donor age, the interval from death to preservation and preservation to surgery, and the amount of endothelial cells in the donor cornea were used to evaluate the clinical result.

STATISTICAL METHODS

SPSS version 16 (SPSS Inc, Chicago, IL, USA) was used to conduct the statistical analysis. Using the Wilcoxon signed rank test, differences between preoperative and postoperative values in the same group were examined. For intergroup comparisons, the Mann-Whitney U test was applied. Statistical significance was defined as a "p" value 0.05.

TABLE 1: Demographic properties of the patients and indications for PK in domestic and imported graft cornea groups.

Characteristics	Domestic group (n=15)	Imported group (n=13)	p value
Gender (Female/Male)	9/6	7/6	0.74
Age ($\bar{X}$±SD) (years)	58.53±9.74	59.08±7.96	
(range)	(38-72)	(42-70)	0.87
Laterality (right/left eye)	7/8	6/7	0.98
Indications for PK			
Keratoconus	3	3	0.35
Graft failure	2	3	
Corneal dystrophy	2	2	
Corneal scar due to herpetic keratitis	2	2	
Corneal scar due to perforatingeye injury	4	3	
Corneal leukoma	2	-	
Nuclear opalescence (Grade 2/3)	8/7	6/7	0.705

PK: Penetrating keratoplasty; SD: Standard deviation.

RESULTS

This study included 28 eyes of 28 cases who underwent cataract extraction with phacoemulsification following PK with domestic and imported corneas. Table 1 lists the demographic characteristics and indicators for PK. Regarding age (p=0.87), gender (p=0.74), laterality (p=0.98), PK indication (p=0.35), and nuclear opacity (p=0.71), there were no discernible differences between the groups. The time interval between PK and cataract extraction was 18.6±6.18 (11-35) months at domestic group and 19±5.24 (12-31) months at imported group (p=0.86). Table 2 displays the preoperative donor characteristics. The domestic group had a lower donor age (p=0.049), shorter preservation-to-surgery times (p<0.001) and higher ECD (p<0.001).

The number of endothelial cells in the donor corneas is shown in Table 3 and the percentage of hexagonality in the donor corneas is shown in Table 4. The ECD was significantly higher in domestic donor corneas compared to imported donor corneas before PK, before cataract surgery, and at all follow-up examinations after cataract surgery (p<0.05, for each). The percentage of mean decrease in the number of endothelial cells was 16.41±3.89% preoperative to postoperative 1st month in the domestic group and 16.46±2.70% in the imported group (p=0.98), 6.47±3.29% postoperative 1st to 3rd month in the domestic group and 10.32±2.17% in the imported group (p=0.001), 6.33±2.73% for the 3rd to 6th month in the domestic group and 9.25±2.53% in the imported group (p=0.007) and 7.53±3.63% for the 6th to 12th month in the domestic group and 11.79±1.76% in the imported group (p=0.001). The percentage of the hexagonality in both groups was not statistically different pre- and post-cataract surgery (p>0.05, for each).

During cataract surgery, the sutures of 3 patients in the domestic group and 2 patients in the imported

TABLE 2: Donor-related factors.

Characteristics	Domestic group (n=15)	Imported group (n=13)	p value
Age ($\bar{X}$±SD) (years) (range)	54.47±11.15 (29-65)	60.85±3.13 (56-65)	0.049
D-P time ($\bar{X}$±SD) (hours) (range)	6.53±2.29 (1-17)	7.00±3.14 (1-22)	0.66
P-O time ($\bar{X}$±SD) (day) (range)	2.87±1.46 (1-8)	5.38±1.04 (3-8)	<0.001
Endothelial density ($\bar{X}$±SD) (cells/mm^2) (range)	2910.73±312.16 (2451-2454)	2463.54±236.99 (2043-2876)	<0.001

SD: Standard deviation; D-P: Death-to-preservation; P-O: Preservation-to-operation.

TABLE 3: The change of mean endothelial cell count in domestic and imported graft cornea groups.

	Domestic group (n=15)	Imported group (n=13)	p value
Endothelial cell density ($\bar{X}$±SD) (cells/mm^2) (range)			
At donor cornea before PK	2910.73±312.16 (2451-2454)	2463.54±236.99 (2043-2876)	<0.001
At graft before cataract surgery	2528.26±275.49 (2177-3197)	2170.85±196.65 (1835-2531)	0.001
Postoperative 1st month	2115.83±275.07 (1762-2705)	1812.43±161.41 (1596-2132)	0.002
Postoperative 3rd month	1984.16±308.80 (1620-2674)	1625.57±154.65 (1447-1985)	0.001
Postoperative 6th month	1854.54±260.35 (1530-2413)	1474.71±139.57 (1303-1796)	<0.001
Postoperative 12th month	1717.72±223.69 (1433-2185)	1300.34±118.05 (1148-1555)	<0.001

SD: Standard deviation; PK: Penetrating keratoplasty.

TABLE 4: The change of the hexagonalite in domestic and imported graft cornea group.

	Domestic group (n=15)	Imported group (n=13)	p value
Percentage hexagonality (%)			
Preoperative	53.33±7.60 (43-65)	51.54±8.79 (41-65)	0.57
Postoperative 1st month	50.60±7.54 (40-62)	48.46±8.73 (39-60)	0.49
Postoperative 3rd month	48.33±7.84 (37-60)	45.69±8.62 (36-58)	0.41
Postoperative 6th month	47.00±7.82 (36-58)	44.38±8.56 (35-57)	0.41
Postoperative 12th month	46.00±7.44 (36-57)	42.85±8.99 (30-55)	0.33

group were still present. After cataract surgery, suture loosening, elevated intraocular pressure, and herpetic epithelial keratitis recurrence was observed in one patient in each group. Newly acquired herpetic epithelial keratitis was observed in 2 patients in the domestic group and in 1 patient in the imported group. Loose sutures in 2 eyes were removed, as it was more than 1 year since the PK. Elevated intraocular pressure and herpetic epithelial keratitis were controlled with medical treatment. No graft rejection reaction or failure developed in any donor cornea after cataract surgery. Graft survival was 100%.

DISCUSSION

The availability of donor tissue is a significant issue in Türkiye, as it is in all other nations. International donor cornea sharing has become possible as a result of reports of only a slight loss of endothelial cells occurring when the donor cornea is stored at 4 °C for up to 14 days in a chondroitin sulfate solution.[12]

Surgical trauma, a change in endothelial cell distribution, and allograft rejection are reasons for endothelial cell loss after PK and the cumulative rate of loss has been reported to be 33% in the first 2 years and ≥50% in the first 10 years.[3,4,6,8,13] Cataract surgery in keratoplasty eyes constitutes a risk for graft survival where endothelial cell loss is already present. Cataract surgery after PK is quite safe because of closed-system surgery, but complications such as endothelial cell loss during surgery, graft rejection, glaucoma and infection after surgery may be seen. There are several studies in literature which have compared combined and sequential surgeries and the techniques used for cataract extraction in respect of endothelial cell loss.[9,10,13-16] According to Hayashi and Hayashi combined and sequential operations resulted in the same amount of endothelial cell loss.[14]

Endothelial cell loss has been reported to be 0.6% per year in healthy eyes and 2.5% per year after cataract surgery.[7,8] It has been reported that ECD after cataract surgery in keratoplasty eyes decreased more compared to preoperative eyes without keratoplasty, and that the decrease was most frequently in the first 2 years postoperatively.[15,16] According to the study by Kim and Kim, following phacoemulsification, transplanted corneas lost more endothelial cells than normal corneas.[15] After a 6-month follow-up period, Hsiao et al. reported no discernible difference in ECD after intraocular lens implantation in transplanted corneas.[16]

While endothelial cell preservation against aging and further surgeries is the most crucial element in the long in long-term follow-up, endothelial cell count is the primary parameter impacting graft survival in the early postoperative period. A previous study by the current authors indicated that although domestic corneas exhibited a much higher ECD in the preoperative and postoperative periods, this difference was not found to influence the graft survival.[5] According to our knowledge this study is the first to investigate how the graft type utilized in PK eyes affects corneal ECD following cataract surgery in eyes with transplanted corneas. In eyes that have previously undergone PK, cataract surgery causes a loss of endothelial cells at a rate of 44.89% after a year and 58.10% after two years.[6-8] In the current study, the average rate of endothelial cell loss was 30% in the domestic group and 40% in the imported group. The reason why the endothelial loss is so high in the first year is the addition of endothelial cell damage due to phacoemulsification during surgery and inflammation secondary to surgery to the endothelial cell loss already present after keratoplasty. The postoperative 12-month (short-term) results in this study show that domestic donor corneas had a significantly higher ECD in the preoperative and postoperative periods compared to the imported group. After cataract surgery, the number of endothelial cells decreased progressively throughout the follow-up period in both groups. Except for the preoperative and postoperative 1st month, the percentage of mean decrease in the number of endothelial cells was statistically higher in the imported group at the other time-points examined, but this difference in the number of endothelial cells and the percentage of decrease rate between the two groups did not affect graft survival. Eyes with graft failure and corneal scarring due to herpetic keratitis and perforated eye injury who underwent PK are more prone to inflammation. In this current study, the eyes with these indications were in similar numbers in both groups, and before cataract surgery, patients were carefully examined in terms of conditions that may trigger inflammation (such as corneal vascularization, glaucoma, herpetic keratitis). These factors explain why graft survival is high in the 12-month period after cataract surgery.

There were numerous drawbacks to this study, chief among them the limited sample size and the fact that the data only represent one center. The results were based on the short-term follow-up of patients and did not take into account the non-uniform distribution of PK indications. The plateau in the curve of endothelial cell loss in these eyes may warrant further investigation, as previously suggested.

CONCLUSION

In conclusion, since cataracts develop due to both aging and the drugs used in patients who have undergone PK, cataract surgery is the most common post-PK surgery. Although the imported donor corneas were older, had a longer preservation-to-surgery time and a lower ECD, and higher postoperative ECD loss rates the graft survival rates are similar. Thus, although the preoperative and postoperative endothelial characteristics are worse in imported cornea group than the domestic donor group this difference does not lead to a difference in graft survival rates up to one year. In light of this, the global cornea sharing system appears to be a dependable alternative for nations with a limited supply of donor tissue. Additional studies with longer follow-up times are required.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Evin Şingar, Ayşe Burcu;* ***Design:*** *Evin Şingar, Barış Oral, Selma Özbek Uzman;****Control/Supervision:*** *Ayşe Burcu, Evin Şingar;* ***Data Collection and/or Processing:*** *Evin Şingar, Barış Oral;* ***Analysis and/or Interpretation:*** *Evin Şingar, Züleyha Yalnız Akkaya;* ***Literature Review:*** *Evin Şingar, Barış Oral;* ***Writing the Article:*** *Evin Şingar;* ***Critical Review:*** *Züleyha Yalnız Akkaya, Ayşe Burcu.*

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