

Clinical Effects of Donor Cornea Variables on Outcomes After Deep Anterior Lamellar Keratoplasty: A Retrospective Research

Donör Kornea Değişkenlerinin Derin Ön Lamellar Keratoplasti Sonrası Sonuçlar Üzerine Klinik Etkileri: Retrospektif Bir Araştırma

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ABSTRACT Objective: Evaluating the clinical effects of donor cornea variables on outcomes after deep anterior lamellar keratoplasty (DALK). **Material and Methods:** In this retrospective study, 44 eyes of 44 patients who underwent DALK using the Anwar's big bubble technique for different corneal pathologies at the ophthalmology department of Haydarpaşa Numune Training and Research Hospital were included. Preoperative and postoperative best corrected distance visual acuity (BCDVA), slit-lamp examination and complications were recorded. Postoperative refractive error, keratometry and spherical equivalent refractive error were recorded if they could be measured. Corneal wavefront aberration analysis was evaluated in 8 eyes. Donor data including age, death-to-preservation (DP) time and preservation-to-surgery (PS) time were evaluated. **Results:** The mean patient age was 35.06±16.48 (range 15-75) years, there was a significant increase in postoperative BCDVA (range 0-1.09 logMAR) in all eyes ($p<0.001$). Donor age (range 18-75 years), donor endothelial cell density (range 1,739-2,456) and DP time (range 15-1,080 minutes) were not significantly associated with postoperative best corrected visual acuity, keratometric astigmatism (range 0.50-9.75), refractive error (range -9.0-7.75), spherical equivalent refractive error (range -0.38-9.38) and corneal wavefront aberration analysis. PS time (range, 1-10 days) was correlated with postoperative corneal astigmatism ($r=0.417$; $p<0.05$). Donor age and postoperative spherical refractive error were statistically correlated in the negative direction ($r=-0.504$; $p<0.05$). **Conclusion:** PS time was correlated with keratometric astigmatism value in the positive direction, and donor age was negatively correlated with postoperative spherical refractive error. Other factors, including DP time and graft endothelial cell count were not statistically associated with postoperative outcomes.

ÖZET Amaç: Bu çalışmanın amacı, donör kornea özelliklerinin, derin anterior lameller keratoplastinin [deep anterior lamellar keratoplasty (DALK)] sonuçlarına etkisini değerlendirmektir. **Gereç ve Yöntemler:** Farklı tanılarla Haydarpaşa Numune Eğitim ve Araştırma Hastanesi Göz Kliniğinde Anwar'ın büyük hava kabarcığı tekniği ile DALK yapılan 44 hastanın 44 gözü retrospektif olarak değerlendirildi. Preoperatif ve postoperatif düzeltilmiş en iyi görme keskinliği (DEİGK), postoperatif keratometri değerleri, refraksiyon kusuru, sferik eş değerleri ve karşılaşılan tüm komplikasyonlar değerlendirilerek kaydedildi. Takip edilen 8 hastanın sütür alımı sonrası korneal wavefront aberasyon analizi yapıldı. Korneası alınan donörün yaşı, korneanın endotel yoğunluğu, donörün ölümü ile korneanın alımı ve alımından nakline kadar geçen saklama süresi çalışmamızda değerlendirmeye alındı. **Bulgular:** Çalışmaya alınan hastaların yaş ortalaması 35,06±16,48 (15-75) yılı. Kırk dört gözün tümünde postoperatif DEİGK (0-1.09 logMAR) artmış izlendi. Donör yaşı (18-75 yıl), donör kornea endotel yoğunluğu (1.739-2.456), donörün ölümü ile korneanın alımı arasındaki süre (15-1.080 dk) ile postoperatif DEİGK, keratometrik astigmatizma (0,50-9,75), refraksiyon değeri (-9,0-7,75), sferik eş değer (-0,38-9,38) ve korneal wavefront aberasyon analizi ölçümleri arasında istatistiksel olarak anlamlı ilişki saptanmadı. Kornea alım-cerrahi zamanı (1-10 gün) ile postoperatif keratometrik astigmatizma ölçümleri arasında istatistiksel olarak anlamlı ilişki saptandı ($r=0,417$; $p<0,05$). Donör yaşı ile postoperatif sferik refraksiyon kusuru arasında negatif yönde istatistiksel olarak anlamlı korelasyon izlendi ($r=-0,504$; $p<0,05$). **Sonuç:** Donör kornea alım ve cerrahi zamanı arasındaki sürenin keratometrik astigmatizma ile pozitif yönde, donör yaşının ise postoperatif sferik refraksiyon kusuru ile negatif yönde korele olduğu saptandı. Donör kornea endotel sayısı ve ölüm ile donör kornea alım arasındaki sürenin ise postoperatif klinik sonuçlar ile istatistiksel olarak anlamlı ilişkisi gözlenmedi.

Keywords: Corneal transplantation; eye banks

Anahtar Kelimeler: Korneal transplantasyon; göz bankası

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Peer review under responsibility of Türkiye Klinikleri Journal of Ophthalmology.

Received: 12 Jul 2021

Received in revised form: 25 Oct 2021

Accepted: 18 Nov 2021

Available online: 26 Nov 2021

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Corneal transplantation is the main treatment for patients with corneal blindness. The Eye Bank Association of America statistical report which was revealed in 2019 showed that full thickness grafts are being used more than 90% for surgical treatment of corneal diseases.¹ In recent years, lamellar techniques which selectively replace only the pathologic layers of the cornea have been developed. Deep anterior lamellar keratoplasty (DALK) is a lamellar corneal graft procedure which can be performed in patients with healthy endothelium. Although DALK preserves healthy corneal tissue therefore has lower endothelial rejection rate, it wasn't widespread because it has a steep learning curve, longer surgery time and interface problems due to manual lamellar dissection. With the improvement in surgical instruments and new techniques for lamellar dissection, studies have reported visual outcomes comparable with full thickness corneal transplant. Lamellar keratoplasty is now the most preferred treatment choice for patients with healthy Descemet's membrane (DM) and endothelium.

DALK has undisputed advantages compared to penetrating keratoplasty (PK). Preserved endothelium allows lower rates of allograft rejection. The structural integrity of the eye during the surgery is maintained hence less complications develop. Reduced usage of postoperative steroids provides better healing of the corneal wound and there is less risk of secondary glaucoma. In their study Arundhati et al. compared the long-term graft survival rates between PK and DALK and reported that PK has higher risk of late postoperative complications, graft rejection and glaucoma.² In DALK, donor endothelial cell count is not an important factor for donor cornea use in contrast to PK, so it improves donor cornea accessibility where there is insufficient donor supply. It is known that there is a universal shortage of donor corneas available for transplant and many of them are unsuitable due to low endothelial cell count.³ With the use of only the stroma of donor cornea, DALK gives the opportunity to use donor corneas with lower endothelial cell count and longer preservation time.

According to Eye Bank Association of America standards for human corneal transplantation, limits of donor age, minimal endothelial cell count and death-

to-preservation (DP) time are left to the preference of the eye banks.⁴ Studies assessing the impact of donor and eye bank variables on the quality of donor corneas and clinical outcomes assist to establishment of eye banking standards. The present study aimed to evaluate the impact of donor and eye bank variables on the quality of donor corneas, postoperative complications, visual and refractive outcomes of deep lamellar keratoplasty.

MATERIAL AND METHODS

In this retrospective study, records of patients who underwent DALK from January 2010 to November 2015 at the Cornea Clinic of the Ophthalmology Department at Haydarpaşa Numune Training and Research Hospital, İstanbul, Turkey were compiled. All the protocols of our study were concordant with the tenets of the Declaration of Helsinki, and informed consent was obtained from all patients. Ethical approval for this study was obtained from Haydarpaşa Numune Training and Research Hospital Ethics Committee (HNEAH-KAEK: 2015/265, 14.12.2015).

Patients who underwent DALK using Anwar's big-bubble technique and were observed for more than 6 months postoperatively were included in the study. The exclusion criteria were the following; co-existence of other ocular pathologies such as cataract, retinal disorders and glaucoma, lack of sufficient donor data, a follow-up time less than 6 months. Patients who had other ocular surgery combined with DALK, who had an ocular surgery before or during the follow-up were excluded from the study.

A complete ophthalmological examination was performed preoperatively, which included measurement of manifest refraction, keratometry, uncorrected and best corrected distance visual acuity (BCDVA), slit-lamp biomicroscopy, Goldmann applanation tonometry, dilated fundus examination and ultrasonography when needed. BCDVA was recorded for evaluation.

DONOR PREPARATION AND EXAMINATIONS

All donor corneas were obtained from the eye bank of Haydarpaşa Numune Training and Research Hospital, Ophthalmology Clinic. They were taken as a sele-

rocorneal button from a cadaver according to standard eye bank rules. Storage of the donor tissue was in Corneal Chamber (Alchimia, Italy) solution at 4 °C. Eye bank data including age and sex of the donor, DP time (minutes), preservation-to-surgery (PS) time (days), and graft rating were recorded. The central corneal endothelium was evaluated using a specular microscope (Konan Eye Bank Kerato Analyzer, Konan. Medical Inc., Japan) before preservation.

SURGICAL TECHNIQUE

The surgical procedures were performed using Anwar’s big-bubble technique under general anesthesia. Horizontal corneal diameter and the location of the cone determined the size of trephination. A Hessburg-Barron vacuum trephine (range, 7.00 to 8.00 mm in diameter) was used for partial-thickness trephination (60% to 80% corneal depth). With a 30 G needle, air was injected and a large air bubble which showed complete separation of DM by reaching the trephination line was formed. With a crescent knife, partial thickness keratectomy was performed. The stromal residue tissue was divided into 4 quadrants with blunt Vannas scissors and they were excised for baring DM at the edge of the trephination. After preparing the graft bed, with a vacuum donor punch (Katena Products, Inc. Denville, New Jersey, USA) donor cornea 0.25 mm larger in diameter than the graft bed was obtained. Complete removal of endothelial cells from DM was confirmed with trypan blue dye. The donor cornea was sutured in an interrupted manner using 10/0 nylon sutures. The wound site was closed and sutures were tightened without leakage.

POSTOPERATIVE MANAGEMENT

Postoperatively, all patients received topical moxifloxacin (Vigamox 0.5% sterile ophthalmic solution, Alcon Laboratories Inc., Fort Worth, Texas, USA), prednisolone acetate (Pred Forte 1% sterile ophthalmic solution, Abdi İbrahim, Turkey) and preservative-free artificial tear eye drops 6 times per day for 1 month. Prednisolone acetate 1% eye drops and artificial tears were tapered off over 3 to 6 months depending on the condition of the eye. Patients were examined on postoperative day 1 and subsequently daily until epithelial healing was complete. Follow-up examinations were performed monthly during the

first 6 months and then every 2 months. The follow-up time was 2 years.

Postoperative complications, condition of the donor epithelium and graft clarity were noted at each visit. After removal of the sutures, at the 2nd year examination, refraction was measured and spherical equivalent was calculated. Astigmatism and BCDVA was recorded for each patient. Corneal wavefront aberration measurement (Schwind Sirius Corneal Wavefront Analyzer, Kleinostheim, Germany) was examined in 8 eyes. These postoperative values were compared with donor cornea features.

STATISTICAL ANALYSIS

For statistical analysis, Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) program was used. During study data evaluation, descriptive statistical methods (mean, standard deviation, median, frequency, rate, minimum, maximum) were used. The Wilcoxon signed-ranks test was used for intra-group comparison of non-normal distribution parameters. Spearman’s correlation analysis was used to evaluate the inter-parameter relationships. Significance was evaluated at the levels of p<0.01 and p<0.05.

RESULTS

The study included 44 eyes of 44 patients (25 men, 19 women; mean age, 35.06±16.48 years; range, 15 to 72 years) who underwent DALK. [Table 1](#) summarises the clinical diagnoses of patients included in the study. Factors related to donor cornea are shown in [Table 2](#).

TABLE 1: Indications for keratoplasty in 44 study patients.

		Number of patients
Indication	Granular dystrophy	1
	Postherpetic leukoma	5
	Keratoglobus	1
	Keratoconus	22
	Chemical injury	1
	Leukoma	6
	Macular dystrophy	6
	Pellucid marginal degeneration	1
	Traumatic corneal scar	1

TABLE 2: Data related to donor corneas.

	Mean±SD (minimum-maximum)
Death-to-preservation time (minutes)	382.09±296.47 (15-1,080)
Preservation-to-surgery time (days)	5.38±2.13 (1-10)
Donor age (years)	54.86±12.76 (18-75)
Graft endothelial cell count	2,263.29±113.63 (1,739-2,456)

SD: Standard deviation.

Mean preoperative BCVA was 1.22±0.48 (range 0.50-2.30) logMAR which increased to 0.38±0.27 (range 0-1.09) logMAR at final examination ($p<0.001$). Only 2 of the (4.5%) grafts demonstrated some degree of stromal edema, the remaining grafts were clear on postoperative day 1 and similarly 2 eyes (4.5%) had epithelial defects on day 1, they had complete healing within 2 days after the surgery. Postoperative 4 (9.1%) patients were re-sutured due to loose suture. Peripheral graft vascularization that did not interfere with visual acuity was observed in 1 (2.3%) eye. Only 1 eye presented with formation of a double anterior chamber despite the apparent absence of an intraoperative DM rupture, it spontaneously resolved during follow-up. Graft rejection was not experienced and at the final follow-up examination, all the grafts remained clear.

Postoperative keratometric astigmatism values, spherical refractive error, spherical equivalent refractive error of 44 eyes and trefoil aberrations, coma aberrations and total root mean square (RMS) values of 8 eyes were evaluated. The values are shown in Table 3. These postoperative values and donor cornea characteristics are compared in Table 4.

Postoperative best corrected visual acuity, keratometric astigmatism, spherical equivalent, astigmatism, trefoil aberration, coma aberration and total RMS measurements were not correlated with donor age, graft endothelial cell count and DP time ($p>0.05$).

There was a statistically significant correlation between PS time and postoperative keratometric astigmatism measurements at the level of 41.7% ($r=0.417$, $p=0.013$, $p<0.05$) in the positive direction (increasing keratometric astigmatism measurement as the PS time increased). Donor age and postopera-

tive spherical refractive error were statistically correlated at the level of 50.4% ($r=-0.504$; $p=0.012$; $p<0.05$) in the negative direction (decreasing postoperative spherical refractive error as the donor age increased).

DISCUSSION

The present study aimed to evaluate the influence of donor cornea quality and eye bank factors on postoperative complications, visual and refractive outcomes of deep lamellar keratoplasty. Our findings revealed that longer PS time increased keratometric astigmatism value and with increasing donor age, postoperative spherical refractive error decreased. Other factors, including DP time and graft endothelial cell count were not statistically associated with postoperative outcomes. Donor corneas with donor age ≤ 75 years, DP time ≤ 18 hours, endothelial cell densities of $\geq 1,739$ cells/mm² and PS time of ≤ 10 days seem to be safe as a donor tissue for DALK surgery.

While the decrease in endothelial cell count as a result of aging is a common knowledge, recent developments in imaging techniques have also revealed significant changes in other layers.⁵ Increased corneal thickness due to the loss of endothelial cells and epithelial thinning as a result of changes in ocular surface and tear film quality was observed.^{6,7} Confocal microscopic studies showed a significant reduction in keratocyte count and in subepithelial plexus density with increasing age which causes a decrease in corneal sensitivity and delayed wound healing.⁸⁻¹⁰ It has been reported that corneal hysteresis and corneal resistance factors which are evaluated with Ocular Response Analyzer are reduced with increasing age secondary to the

TABLE 3: Postoperative outcomes.

	Mean±SD (minimum-maximum)
Keratometric astigmatism	4.17±2.07 (0.50/9.75)
Spherical refractive error	-0.58±3.47 (-9.0/7.75)
Spherical equivalent refractive error	3.48±2.18 (-0.38/9.38)
Trefoil aberration	0.55±0.22 (0.23/0.93)
Coma aberration	0.45±0.14 (0.24/0.73)
Total root mean square	2.98±1.88 (1.31/6.86)

SD: Standard deviation.

TABLE 4: Comparison of donor cornea characteristics and postoperative outcomes.

Postoperative outcomes		Donor age	Donor endothelial cell count	Death-to-preservation time	Preservation-to-surgery time
BCDVA (logMAR)	n	41	41	41	41
	r value	0.254	-0.055	0.079	0.131
	p value	0.109	0.732	0.622	0.414
Keratometric astigmatism	n	35	35	35	35
	r value	-0.067	0.075	-0.175	0.417
	p value	0.702	0.669	0.316	0.013*
Spherical refractive error	n	24	24	24	24
	r value	-0.504	0.099	-0.056	-0.183
	p value	0.012*	0.647	0.795	0.393
Spherical equivalent refractive error	n	35	35	35	35
	r value	-0.008	-0.109	-0.057	0.177
	p value	0.964	0.534	0.746	0.310
Trefoil aberration	n	8	8	8	8
	r value	-0.619	0.214	0.095	-0.630
	p value	0.102	0.610	0.823	0.094
Coma aberrations	n	8	8	8	8
	r value	0.001	0.371	0.575	-0.677
	p value	1.000	0.365	0.136	0.065
Total RMS	n	8	8	8	8
	r value	0.357	-0.190	-0.119	0.334
	p value	0.385	0.651	0.779	0.419

r value: Spearman's correlation coefficient *p<0.05; BCDVA: Best corrected distance visual acuity; RMS: Root mean square.

loss of collagen fibrils. It has been suggested that these factors are important in donor cornea selection.¹¹

The significance of these changes in donor selection has not yet been clarified. Borderie et al. found that donor age is the only donor corneal factor that affects visual healing. In their study, donor age older than 80 years had a negative effect on postoperative visual acuity.¹² In the present study, there was no significant relationship between donor age and postoperative visual outcomes. However, the mean donor corneal age of our study is 54.86 years which is a relatively young age. This might be a limitation in terms of evaluating the effect of donor corneal age. In addition to the structural changes of the cornea, it is known that with increasing age, refractive changes cause “against the rule” astigmatism. Contrary to our expectations, we found that donor age and postoperative spherical refractive error were correlated in the negative direction. This finding should be confirmed with further studies with a higher number of patients.

Previous studies concluded that longer DP time and PS time increases the incidence of graft epithelial sloughing. In their study, Van Meter et al. evaluated 81 donor corneas before and after PK, it was revealed that the incidence of postoperative epithelial defect is higher in donor corneas with DP time longer than 6 hours.¹³ One study reported that only donor corneal factor statistically related to postoperative epithelial defect is PS time.¹⁴ In their study, Feizi et al. reported that postoperative epithelial defect is correlated with graft quality and donor cornea PS time in patients who underwent DALK.¹⁵ In the present study, postoperative epithelial defect was observed in only 2 eyes and no significant correlation with donor cornea factors was found. In the current study, mean DP time was 382.09 minutes and mean donor cornea PS time was 5.38 days. Studies have shown that donor corneal tissue can be stored safely up to 17 days in the storage solution used in the present study.¹⁶ Early donor cornea removal and early usage of donor cornea in this

study could be a factor for reduced postoperative epithelial defect frequency.

In the current study, no correlation between donor cornea factors, postoperative visual acuity and refractive errors was observed. In a study where donor cornea factors, postoperative best visual acuity and refractive results are evaluated, Feizi et al. reported no correlation between parameters.¹⁵ Similarly, Heindl et al. found that donor PS time and postoperative visual acuity at 1 year after surgery were not significantly correlated.¹⁷ In a study in which the effect of donor cornea characteristics on the corneal graft was evaluated, donor DP time was reported to have minimal effect on postoperative graft clarity, punctate epitheliopathy, and postoperative visual acuity.¹⁸ Observations of Schaub et al. showed no significant association between the donor tissue parameters (donor age, gender, preservation method, donor cornea preservation time, PS time) and the clinical outcome parameters after DALK.¹⁹ In their study, Feizi et al. concluded that duration of donor PS time is correlated with postoperative graft edema and low quality donor graft is correlated with slow resorption of stromal edema.¹⁵ A statistically significant positive correlation between PS time and postoperative keratometric astigmatism measurements was observed in the current study. This can be considered as an effect of edema and as a result increasing thickness of the graft.

In a previous study, postoperative high-order aberrations were found to be high in patients who underwent PK.²⁰ Muftuoglu et al. reported that different curvature, thickness and diameter of donor tissue, wound healing and compression of the sutures are the most important causes of high-order aberrations.²⁰ In the literature, whether DALK causes more high-order aberrations than PK is not clearly assessed in large series and there is no significant difference between the groups.^{21,22} There has not yet been research on the relationship between donor characteristics and aberrations. In the current study, there was no significant relationship between postoperative high order aber-

rations and donor cornea factors. The main limitation of this study is the small number of patients (8 patients). Further studies with increased number of patients are needed to support this result.

Similar to other studies, it was also revealed by the present study, no significant relation was found between donor characteristics, postoperative surgical success and complications. The most important limitation of this study is the small number of patients and the short follow-up period.

CONCLUSION

One of the most important advantages of DALK is the flexibility of donor cornea selection. This study also revealed that the use of donor tissues with a longer DP time and lower quality can provide good refractive outcomes and visual acuity comparable with those achieved after the use of good quality donors.

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: Meltem Toklu, Ece Turan Vural; **Design:** Meltem Toklu, Ece Turan Vural; **Control/Supervision:** Ece Turan Vural, Elvin Yıldız; **Data Collection and/or Processing:** Meltem Toklu, Ece Turan Vural, Elvin Yıldız; **Analysis and/or Interpretation:** Meltem Toklu, Ece Turan Vural; **Literature Review:** Meltem Toklu, Ece Turan Vural; **Writing the Article:** Meltem Toklu, Ece Turan Vural; **Critical Review:** Ece Turan Vural; **References and Fundings:** Ece Turan Vural, Elvin Yıldız; **Materials:** Ece Turan Vural, Elvin Yıldız.

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