

Effect of Donor Graft Quality on Clinical Outcomes After Penetrating Keratoplasty for Keratoconus

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Abstract

Purpose: To evaluate the effect of donor and eye bank characteristics on graft rating and clinical outcomes after penetrating keratoplasty (PK) for keratoconus.

Methods: This retrospective interventional case series included 252 keratoconic eyes which underwent PK. Donor data included age and sex, cause of death, death-to-preservation time, preservation-to-surgery time, epithelial and stromal status, endothelial cell density (ECD) and morphology, and graft rating. Postoperative outcomes included visual acuity, refractive error, epithelial problems, suture-related complications, graft rejection, and graft transparency. Multivariate regression analysis assessed correlations between donor and eye bank characteristics and graft quality, and postoperative outcomes.

Results: Mean recipient and donor age was 29.7 ± 10.0 and 26.2 ± 8.8 years, respectively and mean follow-up period was 66.7 ± 38.5 months. Death-to-preservation time was significantly associated with the presence of graft epithelial sloughing ($P = 0.005$) and stromal cloudiness ($P < 0.001$). Donor age significantly influenced ECD ($P = 0.02$), mean cell area ($P = 0.04$), and hexagonality ($P = 0.01$). The presence of epithelial defects on postoperative day 1 correlated significantly with death-to-preservation time ($P = 0.004$). Graft stromal edema on postoperative day 1 was significantly associated with graft epithelial sloughing ($P < 0.001$). Postoperative visual and refractive outcomes, complications, and graft survival were not correlated with any donor or eye bank factors.

Conclusion: Donor and eye bank variables affected the quality of donor corneas and early postoperative course. However, their long term effect on clinical outcomes, complications, and graft survival were insignificant.

Keywords: Donor Quality; Keratoconus; Penetrating Keratoplasty; Postoperative Outcomes

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INTRODUCTION

Penetrating keratoplasty (PK) is a surgical technique in which the full thickness of the recipient cornea is replaced by donor tissue. PK surgeons prefer to transplant donor cornea tissues of good to excellent quality, which provide adequate endothelial cells for a lifelong period. Donor factors, such as age, local and systemic diseases, cause of death, traumatic damage or surgical procedures, storage factors (mainly storage method), time between death and preservation, and duration of tissue preservation, can influence the final quality of the corneas.^[1-5]

There is currently a paucity of evidence for setting minimum acceptable donor conditions for corneal transplantation. The Eye Bank Association of America standards for human corneal transplantation recommend that minimal endothelial cell count limits, upper and lower limits of donor age, time intervals from death, enucleation or excision to preservation are left to the discretion of eye banks.^[6] An understanding of the effect of donor and eye bank variables, including age, cause of death, time interval from death to enucleation and preservation, storage time, type of storage media, and

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endothelial cell density (ECD) on the appropriateness of corneas for transplantation and post-transplantation outcomes will aid the establishment of eye banking standards. It is vital to identify the correlations between these donor and eye bank parameters and the suitability of corneas for transplantation, and between donor parameters and post-transplantation outcomes to establish the appropriate criteria.

This study investigated the influence of donor and eye bank factors on the suitability of corneas for transplantation and determined whether any of these donor factors affected clinical outcomes, complications, and graft survival following PK in a large group of keratoconic eyes.

METHODS

Records of consecutive patients with keratoconus who had undergone PK from November 1996 to March 2011 were reviewed in this retrospective comparative study for demographic data, pre- and postoperative best-corrected visual acuity (BCVA) and refraction, donor characteristics, time and number of graft rejection episodes, graft clarity, and complications. Keratoconus was diagnosed based on slitlamp findings (corneal ectasia, stromal thinning, Fleischer's ring, and Vogt's striae) and keratometry, and was confirmed by conventional corneal topography.

Inclusion criteria consisted of moderate (mean keratometry 47-52 D) to advanced keratoconus (mean keratometry >52 D or immeasurable keratometry) with poor spectacle-corrected visual acuity, rigid gas permeable (RGP) contact lens intolerance, or inappropriate contact lens fit. Inclusion criteria also required a minimum follow-up duration of 12 months. Exclusion criteria included the co-existence of other ocular pathologies such as vernal keratoconjunctivitis, cataract, retinal disorders and glaucoma or lack of sufficient donor data. Patients who were lost to follow-up were excluded from data analysis. Ethics Committee approval was received to use patients' data.

Preoperatively, a complete ophthalmological examination was performed, which included measurement of uncorrected visual acuity (UCVA), manifest refraction (if possible), BCVA with fully corrective glasses or RGP using a Snellen chart, slit-lamp biomicroscopy, Goldman applanation tonometry, dilated fundus examination, and conventional corneal topography (TMS-1 Topographic Modeling System, version 1.61, Computed Anatomy Inc., New York, USA).

Postoperative outcomes included UCVA, BCVA, manifest refraction, mean keratometry, and keratometric astigmatism measured at the final follow-up examination when no sutures remained. Additionally, postoperative complications, such as epithelial problems, persistent epithelial defects

and suture-related complications, episodes of graft rejection, and graft failure, were noted.

Donor Preparation and Examinations

All donor corneas of good to excellent quality were procured from the Eye Bank of the Islamic Republic of Iran. Enucleation of the eye was performed using standard methods after noting details, such as age, gender, cause of death, history of eye surgery and history of any disease. The whole globe was subjected to gross examination and slit lamp biomicroscopy for grading according to pre-established guidelines. Prerequisites for donor corneas included the absence of any medical or social history factors thought to adversely affect the donor cornea, absence of infectious or structural contraindications, absence of opacification or scar of previous surgeries on slit-lamp examination, death-to-preservation time <48 h, and negative serological results. Matching was not performed between donors and recipients in terms of age and ABO/Rh blood group.

Corneas were preserved at 4°C and supplied as either corneoscleral buttons ($n = 206$, 81.7%) in Optisol medium (Optisol-GS preservative; Chiron Vision, Irvine, CA) or whole globes ($n = 46$, 18.3%) in moist chambers. Eye Bank data included the age and sex of the donor, cause of death, death-to-preservation time (hours), preservation-to-surgery time (days), and graft rating. Eye Bank evaluations of the donor epithelial status, stroma, and ECD and morphology were available for only 99 (39.3%) grafts.

Death-to-preservation time was divided into 2 times intervals (<24 h and 24-48 h) for statistical analyses. Donor epithelial status was graded as intact and sloughing. The severity of epithelial sloughing was graded as mild (epithelial defect less than one-third of the graft), moderate (epithelial defect less than two-thirds of the graft), and severe (epithelial defect more than two-thirds of the graft). The donor stroma status was assigned as clear or cloudy.

The central corneal endothelium was photographed using a specular microscope (Konan Eye Bank Keratoanalyzer, Hyogo, Japan) before preservation. ECD, mean endothelial cell area, coefficient of variation of endothelial cell area, and the percentage of hexagonal cells were calculated from specular photographs. Quality of the donor cornea used for transplantation was graded as excellent, very good, or good based on slit-lamp evaluation of all corneal layers.

Surgical Technique

A single experienced anterior segment surgeon (MAJ) performed all surgeries under general anesthesia using previously described techniques.^[7] The diameter of the recipient trephination was chosen based on vertical corneal diameter (VCD): a 7.75-mm recipient trephine was used for VCD <10.5 mm, and an 8-mm trephine

was used for a VCD ≥ 10.5 mm. The donor cornea was oversized by 0.25 mm in all cases, and was punched from the endothelial side using Barron donor punch (Katena, Denville, New Jersey, USA). The suturing technique (Sharpoint, Angiotech, Vancouver, Canada) included 16-bite interrupted sutures ($n = 38$), one single running suture with 16-18 bites ($n = 171$), or combined 8-bite interrupted accompanied by a 16-bite single running suture, ($n = 43$). Suturing attempted to encompass approximately 90% of the thickness of the recipient and donor tissues. Intraoperative keratometry was performed to adjust suture tension.

Postoperative Course

Patients were examined on postoperative day 1 and subsequently daily until epithelial healing was complete. The status of the donor epithelium and graft clarity was noted at each visit. The corneal surface was carefully examined after application of a fluorescein, and any epithelial defects were recorded. Subsequent follow-up examinations were performed at 1, 3, 6, and 12 months, at least 3 months after complete suture removal and every 6 months thereafter.

All patients received topical chloramphenicol postoperatively every 6 h for 30 days and topical betamethasone 0.1% every 6 h, which was tapered over 2-3 months. Topical hypertonic sodium chloride 5% was prescribed when indicated to reduce graft edema, and topical lubricants were added to hasten epithelial healing. A bandage contact lens (OmniFlex, Hydron, UK) and/or temporary tarsorrhaphy were applied to treat non-healing epithelial defects in cases of persistent epithelial defects (i.e. lasting longer than 14 days).

Keratometric astigmatism was reduced by selective interrupted suture removal starting 3 months postoperatively and adjustments in continuous suture tension. Selective interrupted suture removal was performed sequentially starting with tight sutures (determined by keratometry readings), and was continued until an acceptable amount of astigmatism was achieved. The remaining sutures were left in place unless there was a suture-related complication, such as abscess, loosening, or suture-tract vascularization.

The following factors defined graft rejection reactions: The presence of an epithelial rejection line, presence of subepithelial infiltration, presence of keratic precipitates (KPs) with or without anterior chamber reaction, and graft edema in a previously clear graft with or without KPs or anterior chamber reaction. Edema reversal after corticosteroid administration helped differentiate graft rejection from endothelial decompensation in cases of graft edema without KPs or anterior chamber reaction. Frequent topical betamethasone 0.1% eye drops and occasionally

systemic prednisolone were used to treat acute rejection reactions in corneal transplants.

Graft failure was defined as an irreversible loss of graft clarity from any cause, including persistent epithelial defects, graft rejection, or graft opacity/vascularization.

Statistical Analysis

The data were analyzed using SPSS statistical software version 21 (IBM Corp., Armonk, New York, USA). Normal distribution of continuous variables was verified using the Kolmogorov-Smirnov test and a Q-Q plot. Pre-operative and postoperative visual and refractive outcomes were compared using paired *t*-test and Wilcoxon signed rank test in normal and non-normal continuous variables, respectively. Pearson correlation coefficient evaluated the relationship between normally distributed continuous variables, and Spearman correlation was used for non-normal variables. Multivariate analysis was performed and included variables that were statistically significant at the univariate level ($P < 0.05$). This analysis included a logistic regression of binary and ordinal variables, and a multiple regression of continuous variables. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated from the logistic regression models. Only correlations found statistically significant by multivariate analysis were reported. *P* values < 0.05 were as statistically significant. All reported *P* values are 2-sided.

RESULTS

Recipient and Donor Characteristics

Overall, 252 consecutive (126 right) eyes of 226 (165 male) keratoconus-affected patients underwent PK during the study period. Mean recipient age at the time of surgery was 29.7 ± 10.0 (range, 13-62) years, and mean follow-up period was 66.7 ± 38.5 (range, 12-184) months.

A total of 252 donor buttons from 252 cadavers, including 223 male and 29 female donors with mean age of 26.2 ± 8.8 (range, 7-60) years, were procured. Causes of donor death were multiple trauma in 68.5%, cardiovascular disease in 8.7%, poisoning in 8.2%, suffocation in 6.9%, hanging in 2.6%, internal medical conditions in 1.3%, and other miscellaneous causes in the remaining 3.8% of cases. Maximum storage time was 5 days in optisol medium and 2 days in moist chambers. Donor data are provided in Table 1.

Visual and Refractive Outcomes

Mean preoperative UCVA was 1.53 ± 0.39 (range 0.4-2.60) logMAR which increased to 0.54 ± 0.42 (range 0.05-2.90) logMAR at final examination ($P < 0.001$). Mean preoperative BCVA was 1.34 ± 0.54

Table 1. Data related to donor corneas

Death-to-preservation time (%)	
<24 h	79 (31.3)
24-48 h	173 (68.7)
Preservation-to-transplantation time (days)	1.2±1.1 (0-5)
Graft rating (%)	
Excellent	50 (19.9)
Very good	177 (70.2)
Good	25 (9.9)
Epithelial defects* (%)	
No	33 (33.3)
<30%	40 (40.4)
30-60%	11 (11.1)
>60%	15 (15.2)
Stromal clarity* (%)	
Clear	82 (82.8)
Cloudiness (edema)	17 (17.2)
Endothelial cell*	
Density (cells/mm ²)	3116.9±330.5 (2192-4149)
Mean cell area (µm ²)	322.3±39.0 (241-456)
Coefficient of variation of cell area	34.2±4.5 (22-42)
Hexagonality (%)	57.7±10.1 (38.0-82.0)

*Data relevant to donor epithelial status, stromal clarity, and endothelial cell density and morphology were available in 99 (39.3%) donors

(range 0.10-2.60) logMAR, which improved to 0.17±0.13 (range 0-1.30) logMAR at final follow-up ($P < 0.001$).

Mean preoperative keratometry was 55.05±3.14 D (range 46.0-59.5 D), which was reduced to 45.42±2.54 D (range 38.75-56.00 D) postoperatively ($P < 0.001$). Preoperatively, mean spherical equivalent refractive error and keratometric astigmatisms were -11.84±4.45 D and 5.05±3.26 D, respectively. Postoperatively, these figures decreased to -2.90±3.04 D ($P < 0.001$) and 3.98±2.05 D ($P = 0.01$), respectively.

Postoperative Course and Complications

One hundred and eighty (71.4%) eyes had epithelial defects on day 1. Epithelial defects improved after 5.4±5.8 (range, 1-38) days. A total of 52 (20.6%) eyes had complete epithelial healing within 1-2 days after the surgery. Epithelial cell healing took 3-7 days in 97 (38.5%) eyes. Complete epithelial healing was aided by therapeutic soft contact lenses ($n = 11$) or temporary tarsorrhaphy ($n = 4$), and no grafts failed due to epithelial defects.

A total of 201 (79.8%) grafts were clear on postoperative day 1, and the remaining grafts demonstrated some degree of stromal edema that resolved after 4.6±3.9 days (range, 1-22 days).

Mean time interval from transplantation to first suture removal was 12.2±9.3 months, and the time

from transplantation to complete suture removal was 17.8±8.6 months. Suture-related complications were observed in 76 (30.2%) eyes and included premature loosening or broken sutures ($n = 66$), suture-tract vascularization ($n = 6$) and stitch abscesses ($n = 16$). Sterile stitch abscesses and vascularization of suture tracts were successfully treated with topical corticosteroids and/or suture removal.

Overall, 97 (38.5%) eyes experienced at least one episode of graft rejection, which consisted of epithelial ($n = 3$), subepithelial ($n = 30$), endothelial ($n = 40$), and a combination of subepithelial and endothelial ($n = 24$) rejection. Time interval from transplantation to the first episode of graft rejection was 9.8±16.8 (range 1-115) months. Forty (15.9%) eyes experienced two or more episodes of graft rejection. Frequent topical steroids reversed rejections, and all eyes regained visual acuity just before the rejection episode. At the final follow-up examination, 248 (98.4%) eyes remained clear. Peripheral graft vascularization that did not interfere with visual acuity was observed in four eyes.

Correlations

Death-to-preservation time had a significant positive association with the presence of graft epithelial sloughing ($r = 0.28$, $P = 0.005$, OR = 3.62 [95% CI: 1.30-10.09]) and stromal cloudiness ($r = 0.43$, $P < 0.001$, OR = 10.0 [95% CI: 3.0-33.3]). Donor age significantly influenced ECD ($r = -0.27$, $P = 0.02$, $\beta = -8.8$ [95% CI: -17.3 to -0.4]) and hexagonality ($r = -0.34$, $P = 0.01$, $\beta = -0.51$ [95% CI: -0.85 to -0.17]). Only donor age demonstrated a borderline association with graft rating ($r = -0.17$, $P = 0.05$, OR = 0.96 [95% CI: 0.93-0.99]).

The presence of epithelial defects on postoperative day 1 correlated significantly with graft epithelial sloughing ($r = 0.27$, $P = 0.004$, OR = 2.90 [95% CI: 1.24-6.77]). The time interval from transplantation to complete epithelial healing was influenced by graft stromal cloudiness ($r = 0.24$, $P = 0.03$, $\beta = 4.38$ [95% CI: 0.34-8.43]). Graft stromal edema on postoperative day 1 was significantly associated with graft epithelial sloughing ($r = 0.42$, $P < 0.001$, OR = 0.05 [95% CI: 0.006-0.41]).

Persistent epithelial defects that necessitated bandage contact lens or tarsorrhaphy, suture-related complications, graft rejection episodes and graft clarity at final follow-up examination were not correlated with any donor or eye bank factors. Postoperative BCVA and refractive outcomes were not significantly associated with donor or eye bank variables.

DISCUSSION

This study explored the influence of donor and eye bank characteristics on the quality of grafts used for PK. Our findings revealed that longer death-to-preservation times increased the incidence of graft epithelial

sloughing and stromal edema. Furthermore, donor age negatively influenced ECD and morphology. Other factors, including donor sex, cause of death, and death-to-preservation time, were not significantly associated with endothelial cell features.

Most previous studies that evaluated the effects of donor characteristics and eye bank variables on ECD concluded that donor age and time interval in organ culture were the main variables influencing the quality of the endothelium. Gavrilov et al^[11] reported that the rate of organ-cultured corneas that were inappropriate for PK as a result of inadequate endothelium increased from 13% in donors younger than 40 years to 32% in donors older than 80 years. The Cornea Donor Study revealed a negative correlation between donor age and ECD.^[2] Armitage et al^[3] found that donor age and preservation time in organ culture were the main variables that affected endothelial suitability for PK. The odds of ECD <2500 cells/mm² increased with longer preservation times and increasing donor age.^[3] Increasing time intervals from enucleation to corneoscleral disc excision also increased the likelihood of an ECD <2500 cells/mm², but the overall impact was small and significant only for time intervals >18 h.^[3]

One study measured endothelial cell loss during preservation in organ culture.^[4] Donor's gender, age, cause of death, and postmortem interval were not significantly correlated with the percentage of endothelial cell loss. However, preservation time was significantly correlated with a cell loss of 0.07% for each day of preservation.^[4] We could not evaluate the effect of preservation time on ECD and graft quality because these parameters were measured just before tissue preservation in the present study.

Grabska-Liberek et al^[5] found that morphological ratings of corneas that were suitable for PK depended mostly on the time between death and preservation, donor's age, cause of death and duration of preservation. Only donor age demonstrated a borderline association with graft rating in the present study.

The current study also investigated the influence of donor and eye bank factors, including age, sex, cause of death, time interval from death to preservation, graft quality, storage time and type of storage media, on post-transplantation complications and outcomes. The results demonstrated that epithelial defects on postoperative day 1 correlated significantly with death-to-preservation time. This finding supports the results of previous studies on the effect of donor and eye bank variables on epithelium-related problems following PK. Death-to-preservation time and total storage time were significantly associated with an increased prevalence of epithelial defects on day 1 or hurricane and filamentary keratopathy.^[8,9] Kim et al^[10] outlined that the degree of epithelial defect had a statistically significant association with the

time interval from preservation to surgery. Borderie et al.^[11] reported that death-to-storage time, storage time, and de-swelling time significantly influenced graft re-epithelialization time in univariate analyses. However, multiple regression analyses revealed that none of the donor variables significantly influenced graft re-epithelialization time.^[11]

Graft corneal surface was also a determinant for the success of corneal transplantation during the postoperative period. Corneal epithelial status on day 1 varies from a pristine intact corneal epithelium to a complete epithelial defect with exposure to Bowman's layer. The donor cornea is ultimately resurfaced by recipient's epithelium, but an intact donor epithelium on postoperative day 1 implies a smoother clinical course after corneal transplantation. An unstable graft surface can lead to poor visual acuity because of an irregular tear film interface, discomfort, permanent damage to Bowman's layer, sub-epithelial scarring or infectious keratitis.^[8] Postoperative epithelial problems in the current study were managed by fitting extended-wear soft contact lenses or temporary tarsorrhaphy, and no grafts failed due to epithelial defects.

Despite the effect of donor features on the early postoperative course, other complications, visual and refractive outcomes, and graft survival were not significantly associated with donor or eye bank variables. These observations are consistent with previous reports. Gain et al^[12] found no significant differences in visual acuity and astigmatism with grafts obtained from donors younger than 85 years and donors aged 85 years and older. Halliday and Ritten^[13] found no significant correlation between the time taken to reach a postoperative acuity of 6/12 and donor age.

In the present study, association between baseline donor factors and occurrence of a rejection episode following PK were assessed in the present study in patients with a low-risk condition (keratoconus). Graft rejection was independent of donor tissue characteristics (i.e., age and quality). This finding is consistent with the report by the Cornea Donor Study that reported no correlation between donor age and rejection occurrence.^[4] Another study reported that donor age, ABO compatibility, and other donor factors were not associated with graft rejection.^[15] However, younger donor age was a risk factor for graft rejection (but not graft failure) in three other studies.^[12,16,17]

The majority of studies showed that donor preservation method and time, donor age, cause of death, and preoperative donor ECD and/or morphometric measures (coefficient of variation and hexagonality) did not influence overall graft failure.^[12,16,18-22] Similarly, we found that donor tissue characteristics did not significantly influence graft survival. However, one study reported that preoperative risk factors for the development of late endothelial failure included low

ECD and older donor age.^[23] Authors from the Cornea Donor Study observed that grafts from donors 66 to 75 years old who met the eligibility criteria of their study had a 5-year graft survival rate, which was comparable to grafts from younger donors.^[14] However, higher donor age was significantly associated with lower graft success within a longer follow-up period.^[15] Older donor age and longer storage time are more likely to be associated with lower ECD. In contrast, the results of the current study indicate that as long as the ECD is greater than a given minimum (>2000 cells/mm²) at the time of corneal transplantation, donor parameters will not significantly influence long-term graft survival.

In summary, we investigated factors that influenced donor graft quality and the relationships between donor features and clinical outcomes of PK. The sample we studied was a homogenous group with keratoconus and no other ocular comorbidities, which was suitable for identification of donor features that may influence postoperative outcomes. Our results demonstrated that donor and eye bank variables affected the quality of donor corneas and the early postoperative course. However, the effects on clinical outcomes, complications, and graft survival were insignificant when the minimum selection criteria set by the Eye Bank were respected.

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