

Association between Storage Time of Corneal Grafts and Primary Graft Failure in Penetrating Keratoplasty

Mushafia Hassan¹, Humera Zafar Ali², Sidrah Latif³,
Ramsha Jahangir⁴, Samreen Jamal⁵
^{1,4,5}Mayo Hospital, Lahore,^{2,3}Services Hospital, Lahore

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ABSTRACT

Purpose: To determine association between storage time of corneal grafts and primary graft failure in Penetrating Keratoplasty (PK).

Study Design: Quasi experimental study.

Place and Duration of Study: Institute of Ophthalmology, Eye Unit-III, King Edward Medical University, Mayo Hospital, Lahore from June 2019 to December, 2019.

Methods: In this study, 120 patients (between 11 and 30 years of age) diagnosed with keratoconus were enrolled and divided into two groups. Group A comprised individuals who underwent PK with donor tissues stored for 8 to 14 days, while Group B consisted of patients whose donor tissues were stored for a duration not exceeding 7 days. Patients were followed up for three weeks post-operatively. The data was analyzed using SPSS version 19 and stratified for age, gender and primary graft failure. Post stratification RR was calculated with p-value ≤ 0.05 as significant.

Results: The mean age of patients in Group A was 21.38 ± 6.29 years and in Group B was 19.80 ± 5.81 years. In Group A there were 32 male and 28 female while in Group B there were 29 male and 31 female. In Group A 16(26.7%) patients and in group B, 4 (6.7%) patients had primary graft failure. The risk of primary graft failure was higher in Group A, with RR = 4, p-value < 0.05 .

Conclusion: The risk of primary graft failure in PK is higher when the storage time of corneal grafts is longer. Hence, minimization of storage time may ensure better success rate of corneal grafting.

Key Words: Penetrating Keratoplasty, Corneal Graft, Graft failure. Immuno-suppressants.

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*Correspondence: Humera Zafar Ali
Services Hospital, Lahore
Email: humera.hamid@gmail.com*

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INTRODUCTION

Cornea, being transparent and avascular, is the most successfully transplanted human organ. The principle that explains this high success rate of corneal transplant is based on the avascular nature of the corneal tissue. The quantity of antigen transferred is small and the graft is placed in an avascular bed that checks the immune

system of host from recognizing it.^{1,2} Penetrating Keratoplasty (PK) is performed for keratoconus, Fuchs endothelial dystrophy, bullous keratopathy, keratitis, corneal dystrophies and corneal trauma.³ Both, the surgical and clinical outcome of PK depends upon multiple factors. Surgical outcomes can include primary donor failure whereas clinical outcomes could be early and late postoperative complications or graft rejection altogether. Graft failure is labeled when there is an unrecoverable loss of central graft clarity within early postoperative period, and is determined using a slit lamp biomicroscope.⁴ Primary graft failure may be precipitated by a number of factors. Most common factors include; donor age, cause of death of donor,

cadaver time, donor endothelial cell count, insertion technique, donor diameter, donor thickness and storage time.⁵

It was reported that the patients having corneal transplant with storage time of more than 7 days had 2.4 times greater risk of primary graft failure than those with a shorter storage time.⁶ The ideal storage time varies depending upon the storage technique and storage medium. The Organ Culture method, which is a more elaborate technique, can offer maximum storage time (up to 4-5 weeks), but this technique is both expensive and harder to implement as compared with the Hypothermic method with Optisol (plus/GS), which is most widely practiced, and offers a maximum storage of up to 14 days.⁷

In Pakistan, corneas are imported from Sri Lanka, North America and European countries. Sufficient time usually has already elapsed before transplant, which includes time from cadaver to retrieval, storage and transportation from other countries. This study was conducted to find relation between storage time and primary graft failure in a local set-up in Pakistan.

METHODS

This quasi-experimental study was conducted at Institute of Ophthalmology, Eye Unit-III, King Edward Medical University, Mayo Hospital, Lahore from June, 2019 to December, 2019. A sample size of 120 (60 in each group) was calculated with 80% power of test and taking expected frequency of failure in group A as 20% and group B as 5.5%.⁸ Non-purposive consecutive sampling was done. Patients either male or female, age between 11 and 30 years and diagnosed with keratoconus were enrolled. All other indications of PK including irregular epithelium with advancing epithelial edge, stromal/sub epithelial infiltrates, diffuse endothelial opacities, endothelial rejection line or presence of keratin precipitates were excluded from the study. Informed consent was taken. Patients were divided into two groups (60 patients in each). In group A, patients who underwent PK with donor tissues stored for 8 to 14 days were included and in group B patients with storage time within 7 days were enrolled. Patients were followed up for three weeks post-operatively; first post-op week during the hospital stay and next two weeks on outpatient basis. Topical immunosuppressants were continued according to the standard protocol. Topical steroids (1% Pred Forte eye drops), antibiotics and steroids combination (Tobradex

eye drops) and broad-spectrum antibiotic (Vigamox eye drops) were given every two hours and antiglaucoma beta blockers (Blotim eye drops) twice a day to all the patients and tapered or adjusted according to the clinical status. The final condition of the graft was monitored using slit-lamp biomicroscope at 3 weeks. Primary graft failure was labeled as the presence of following findings on slit lamp examination.

- a) Loss of central clarity.
- b) Presence of Descemet membrane folds.
- c) Central corneal edema (graded as follows).
 - i) Grade 1; corneal haze with striations.
 - ii) Grade 2; corneal haze greater than grade 1 but iris details visible.
 - iii) Grade 3; corneal haze greater than grade 3 with difficult view of iris details.
 - iv) Grade 4; opaque cornea with no anterior chamber view.

The data was analyzed in SPSS version 19. Data was stratified for age, gender, and primary graft failure. Post stratification RR was calculated p-value ≤ 0.05 as significant.

RESULTS

In Group A, there were 25 patients (41.67%) aged 10-20 years and 35 patients (58.33%) aged 21-30 years. In Group B, there were 34 patients (56.67%) aged 10-20 years and 26 patients (43.33%) aged 21-30 years. Among patients aged 10-20 years, the frequency of primary graft failure was higher in Group A (24%) compared to Group B (5.9%), with a relative risk (RR) of 4.08 and a statistically significant p-value of less than 0.05. Similarly, among patients aged 21-30 years, the frequency of primary graft failure was higher in Group A (28.6%) compared to Group B (7.7%), with a relative risk (RR) of 3.71 and a statistically significant p-value of less than 0.05.

Among male cases, the incidence of primary graft failure was higher in Group A, with 9 cases (28.1%), compared to Group B, with 2 cases (6.9%), resulting in a relative risk (RR) of 4.07 and a statistically significant p-value of less than 0.05. Similarly, among female cases, the incidence of primary graft failure was higher in Group A, with 7 cases (25%), compared to Group B, with 2 cases (6.5%), resulting in a relative risk (RR) of 1.34 and a statistically significant p-value of less than 0.05.

Overall, the incidence of primary graft failure in Group A was 16 patients (26.7%), while in Group B it was 4 patients (6.7%), resulting in a relative risk (RR) of 4 and a statistically significant p-value of less than 0.05 (refer to Table 1).

Table 1: Comparison of Primary Graft Failure in Group A and Group B.

Study Groups	Primary Graft Failure		Total
	Yes	No	
Group A	16(26.7%)	44(73.3%)	60(100.0%)
Group B	4(6.7%)	56(93.3%)	60(100.0%)
Total	20(16.7%)	100(83.3%)	120(100.0%)

Relative risk = 4

95% CI = 1.42 - 11.27

p-value = 0.003

DISCUSSION

The primary graft failure (PGF) in our study was 6.7% with storage time of up to 7 days and 26.7% with storage time of 8 to 14 days. Wojcik G et al, found that graft rejection rate in penetrating keratoplasty was not affected by storage time, long transportation route and age of donor.⁸

The inaugural PK was performed by Eduard Zirm in 1905, approximately 120 years ago, resulting in a successful outcome. The avascularity of corneal tissue gives it a low immunological status, hence there is low rejection rate as compared to other organ transplants. Almost 46000 corneal transplants per annum are being performed in United States and so the demand for the donor corneal tissue has increased. Due to the procedural effectiveness and safety, the global demand for corneas exceeds by factor of 70.⁹ Surgeons doing PK in the third world countries have to accept corneas with longer preservation times because of the long travel time.

Corneas from the donors are stored in different medium before they are transplanted. The purpose of storage is to maintain the functional integrity which is essential for a successful transplant.¹⁰

In a study, the median death to transplant time for the corneas stored in McCarey-Kaufman was 1.9 days, in K-Sol was 5.6 days and 24.2 days for organ culture storage. It was concluded that graft failure was not associated with death to transplant time ($P = .07$).¹¹ They compared successful keratoplasties with failed transplants, and found that the increased graft failure

was not associated with donor factors like old age, storage time and death to enucleation time.

Longer graft storage times reduce the endothelial cell density of the donor tissue which leads to corneal de-compensation and primary graft failure. PGF can be due to donor factors such as donor age >70 years, prolonged death to preservation time, prolonged donor storage time, trauma as a cause of donor death, endothelial damage during donor retrieval or storage, and factors relating to surgical technique and surgical trauma.^{12,13,14} Studies have also shown that the graft rejection depended largely on recipient factors and success of graft was limited by complications of immunological rejection, microbial keratitis and glaucoma.^{15,16}

Primary PK survival rate is 81%, in second grafts it is 33% and 16% in third or more grafts. It was 65% for 10-year graft survival rate. It was reported that there was no significance of preservation status ($P=0.096$), time between death and enucleation, time between death and corneal transplant. The authors concluded that the graft failure after PK could be attributed to primary diagnosis and previous graft failures in the recipient.¹⁷

Researchers have been keen on developing ways to overcome the limitation of corneal donation worldwide,¹⁸ to narrow down the gap between supply and demand and reducing the donor cornea wastage. Availability of corneal tissue for longer period of time and planned surgery will open up the long-distance transportation options.¹⁹ Tissue bioengineering for corneal regeneration and stem cell development are the upcoming research projects. In a study by Kinoshita S et al, cultured corneal endothelial cells injection into the recipient's eyes was performed and it was reported to be a successful procedure.²⁰ This technique has a potential to revolutionize the corneal transplantation, but the outcomes require a longer patient follow-up.

Limitation of this study was the small sample size which can be a hurdle to generalizability. Additionally, focusing solely on patients aged 11 to 30 years may not fully represent the broader population with keratoconus. A single-center study may not reflect variations in surgical practices, patient demographics, and post-operative care observed across different healthcare settings, potentially affecting the external validity of the results. Primary graft failure was the primary outcome measure in this study; however, other important clinical endpoints, such as visual acuity outcomes, graft rejection rates, or endothelial cell counts, were not

evaluated, limiting the comprehensiveness of the study findings.

Addressing these limitations in future research could enhance the validity and applicability of the findings in guiding clinical decision-making for patients undergoing PK.

CONCLUSION

There is a high risk of primary graft failure in PK when the storage time of corneal grafts is longer. Hence, minimization of storage time may ensure better success rate of corneal grafting.

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Patient's Consent: Researchers followed the guidelines set forth in the Declaration of Helsinki.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval: The study was approved by the Institutional review board/Ethical review board (892/RC/KEMU).

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Authors Designation and Contribution

Mushafia Hassan; Medical Officer: *Concepts, Design, Literature search, Data acquisition.*

Humera Zafar Ali; Associate Professor: *Design, Literature search, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing Manuscript review.*

Sidrah Latif; Assistant Professor: *Manuscript preparation, Manuscript editing Manuscript review.*

Ramsha Jahangir; Senior Registrar: *Manuscript preparation, Manuscript editing Manuscript review.*

Samreen Jamal; Senior Registrar: *Manuscript preparation, Manuscript editing Manuscript review.*

