Ophthalmology Section

Impact of Donor Age and Cornisol Storage Duration on Endothelial Cell Count and Morphology in Donor Corneal Tissues: A Prospective Observational Study

ABHAY A LUNE¹, PRADIPTA POTDAR², PRANAV MORE³, SHIKHA RAI⁴



ABSTRACT

Introduction: Corneal transplantation remains one of the most successful forms of solid tissue transplantation and a crucial treatment modality for visual rehabilitation in patients with corneal blindness. The success of keratoplasty is largely determined by the health of the corneal endothelium, a non regenerating monolayer crucial for maintaining corneal clarity. Factors such as donor age, cause of death and storage duration influence endothelial viability, especially in intermediate-term storage media such as Cornisol, widely used in Indian eye banks.

Aim: To study and serially evaluate the donor corneal Endothelial Cell Count (ECC) and morphology during Cornisol storage, in association with donor age and cause of death.

Materials and Methods: This prospective observational study was conducted at a tertiary care hospital and research centre, Pune, Maharashtra, India which included 75 donor corneoscleral rims stored in Cornisol between September 2023 and March 2025. Seventy-five donors were categorised into three age groups: Group I (0-30 years), Group II (31-60 years), and Group III (61-90 years). Specular microscopy was performed on days 0, 1, 3, 5, 7, 10 and 14, or until the end of storage. ECC, hexagonality (%HEX), and Coefficient of Variation (CV%) were analysed over time using repeated-measures Analysis of Variance (ANOVA)

for intragroup comparisons, while one-way ANOVA followed by Tukey's post hoc test was applied for intergroup comparisons. A p-value <0.05 was considered statistically significant.

Results: Group I (0-30 years) had the highest baseline ECC and the lowest percentage ECC loss (7.8% by day 5). Group II (31-60 years) showed the greatest ECC loss (36.5% by Day 14), followed by Group III (61-90 years) with 30.4% loss. Intragroup ECC decline was statistically significant in all groups (Group I: p-value=0.009; Groups II and III: p-value <0.001). Intergroup differences in ECC, hexagonality and CV were also significant across most time points (p-value <0.001, except day 14 for ECC where p-value=0.2206). Percentage ECC loss was highest in corneas from trauma-related deaths (45.3%), followed by cardiorespiratory failure (41.6%) and non traumatic intracranial haemorrhage (22.4%), with statistically significant differences across most time points (except day 5, p-value=0.071).

Conclusion: Younger donor corneas demonstrated superior ECC and stability of morphometric parameters. Significant deterioration was noted in older donor tissues, especially beyond day 5 of storage. Donor age, storage duration and cause of death all influenced endothelial viability, supporting early use and careful donor selection.

Keywords: Corneal graft survival, Donor selection, Eye banks, Specular microscopy techniques, Tissue preservation

INTRODUCTION

Corneal transplantation remains one of the most successful forms of solid tissue transplantation and a crucial treatment modality for visual rehabilitation in patients with corneal blindness [1]. The availability and quality of donor corneas continue to be a major limiting factors in meeting this demand. Among the determinants of graft success, the functional integrity of the donor endothelium plays a critical role due to its inability to regenerate. The corneal endothelium is responsible for maintaining stromal deturgescence and optical clarity. Parameters such as ECC, hexagonality (%HEX), and CV% provide crucial insight into endothelial health and are now standard tools in pretransplant tissue evaluation. Loss or dysfunction of endothelial cells beyond a critical threshold can result in irreversible corneal oedema and graft failure [2].

Bourne WM and Kaufman HE, documented age-related endothelial changes including pleomorphism and polymegathism [1]. Matsuda M et al., also reported a reduction in ECC and increased morphological abnormalities with age in healthy corneas [3], and Krohn J and Høvding G further highlighted the impact of donor age and cause of death on endothelial integrity [4]. Armitage WL and Easty DL, emphasised the detrimental influence of systemic conditions on corneal graft survival [5]. Patel SV et al., found

increased endothelial loss in recipients of older donor tissues, underlining the importance of stratifying donor selection by age [6]

Storage conditions also significantly influence endothelial survival. Mehta JS and Tan DT, discussed donor-related factors impacting keratoplasty outcomes [7]. Jain P and Joshi A, reported a consistent decline in ECC with increasing donor age [8]. Indian eye banks predominantly use Cornisol due to its affordability and intermediate-term preservation properties. Rao SK et al., demonstrated that donor age and endothelial quality significantly influence tissue utilisation in Indian settings [9]. Chauhan R et al., conducted assessments of endothelial parameters in Cornisol-stored corneas and recommended utilisation within seven days [10]. Gain P et al., provided global insights on eye banking practices [11], while Sharma N et al., compared Cornisol with Optisol-GS, finding the former effective for short-term preservation up to seven days [12].

However, there is a paucity of Indian studies that evaluate endothelial health at serial time points during Cornisol storage while stratifying by age and cause of death. Most existing studies have either relied on single time-point evaluations or lacked correlation with morphometric indices [3,4,8,10]. This study addressed that gap by prospectively evaluating endothelial cell parameters at multiple time points during

Cornisol storage and associating these changes with donor age and cause of death. The novelty of this study lies in its granular, serial analysis of endothelial degradation over time, its relevance to intermediate-term storage conditions commonly used in India, and its aim to inform evidence-based tissue allocation policies. In doing so, it provides a foundation for optimising donor selection and improving transplant outcomes in resource-constrained settings.

MATERIALS AND METHODS

This was a prospective observational study conducted at a tertiary care hospital and research centre in Western Maharashtra, India between September 2023 and March 2025. The study was approved by the Institutional Ethics Committee (IEC Approval No: IESC/PGS/2023/112).

Inclusion criteria: Donor corneal tissues if stored in Cornisol medium and showed no clinical signs of epithelial or stromal pathology were included in the study.

Exclusion criteria: Tissues with a history of corneal oedema, exposure keratopathy, globe rupture, enucleation, or if they were not stored in Cornisol. Corneas with inadequate specular microscopy imaging or poor-quality central images were also excluded from the study.

Sample size estimation: The sample size was calculated based on prior data assuming a medium effect size for differences in endothelial parameters between donor age groups, with a power of 80% and a significance level of 5% (α =0.05). Using these assumptions, the minimum required sample size was estimated to be 69. To account for potential exclusions and image acquisition failures, a total of 75 donor corneas were enrolled. Based on feasibility, the study included all samples received in the Department of Ophthalmology in a tertiary hospital and research centre in Western Maharashtra from September 2023 to March 2025.

Study Procedure

Donor corneas were harvested from hospital-based deceased individuals after obtaining appropriate consent. Standard sterile enucleation protocols were followed and tissues were immediately transferred into Cornisol medium and stored at 4-8°C. The cold chain was maintained during transport using insulated cool boxes with ice packs.

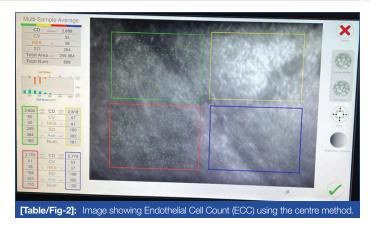
Specular microscopy was performed using the Konan CellChekD non contact microscope [Table/Fig-1] using the centre method [Table/Fig-2]. After equilibrating each cornea to room temperature (approximately 30 minutes), central endothelial images were obtained. Evaluations were carried out serially on days 0, 1, 3, 5, 7, 10, and 14, or until the tissue was distributed.



[Table/Fig-1]: Image showing Konan CellChekD non contact microscope.

Parameters analysed included:

ECC in cells/mm²



- Hexagonality (%HEX), representing the percentage of six-sided cells
- CV%, reflecting cell size variability

Donor data including age, cause of death and time since storage were recorded. Donors were stratified into three age groups: group I (0-30 years), group II (31-60 years), and group III (61-90 years).

STATISTICAL ANALYSIS

Data were compiled and analysed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Quantitative variables such as ECC, hexagonality (%HEX) and CV% were expressed as means±standard deviations. Intergroup comparisons across the three age groups (Group I, II, III) were performed using one-way ANOVA from Day 0 to Day 5. Where significant differences were found, Tukey's post hoc test was applied for multiple pairwise comparisons. For days 7, 10, and 14, due to the absence of data in group I, intergroup comparisons were limited to group II and group III and were assessed using independent t-tests. A p-value <0.05 was considered statistically significant for all analyses.

RESULTS

A total of 75 donor corneas were included in the study. The distribution of donor tissues by age is shown in [Table/Fig-3]. The most common causes of death were cardiorespiratory failure in 38 donors (50.7%) [Table/Fig-4].

Age of the donor (in years)	n (%)
Group-I (0-30)	11 (14.7)
Group-II (31-60)	50 (66.7)
Group-III (61-90)	14 (18.7)
[Table/Fig-3]: Age distribution	

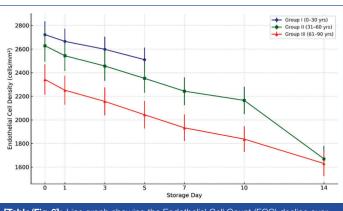
Cause of death	n (%)
Cardiorespiratory failure	38 (50.7)
Non traumatic causes with intracranial haemorrhage	27 (36)
Traumatic causes with intracranial haemorrhage	10 (13.33)
[Table/Fig-4]: Cause of death	

Endothelial Cell Count (ECC) changes over time

Baseline ECC was highest in group I (2722.55±112.4 cells/mm²), followed by group II (2628.41±134.6 cells/mm²) and group III (2342.29±128.7 cells/mm²) [Table/Fig-5,6]. Intragroup ECC decline was gradual in group I (p-value=0.009), moderate in group II (p-value <0.001), and most pronounced in group III (p-value <0.001). By day 14, group II showed the highest percentage ECC loss (36.5%), followed by group III (30.4%), while group I showed only 7.8% loss by day 5, beyond which data were unavailable. Intergroup comparisons at each time point showed statistically significant differences from day 0 to day 10 (p-value <0.001) except on day 14 (p-value=0.2206). Tukey's post-hoc test indicated significant

Age group (in years)	Day 0 Mean±SD	Day 1 Mean±SD	Day 3 Mean±SD	Day 5 Mean±SD	Day 7 Mean±SD	Day 10 Mean±SD	Day 14 Mean±SD	Mean % ECC Loss±SD	Intragroup p-value
0-30	2722.55±112.4	2665.12±108.3	2598.34±105.7	2510.23±102.9	-	-	-	7.8±2.4%	0.009
31-60	2628.41±134.6*	2543.67±129.2*	2456.89±125.8*	2351.76±121.4*	2243.12±117.6#	2165.34±114.2#	1670.12±110.5	36.5±4.7%	<0.001
61-90	2342.29±128.7*	2251.43±122.5*	2156.71±118.9*	2043.56±115.2*	1932.84±111.7#	1837.29±108.3#	1630.67±106.9	30.4±4.2%	<0.001
Intergroup p-value	<0.001	<0.001	<0.001	<0.001	<0.001‡	<0.001‡	0.2206 [‡]	-	-

[Table/Fig-5]: Table showing the Endothelial Cell Count (ECC) decline over time stored in cornisol media.
*Statistically significant difference compared to Group-I (0-30 years) (Day 0-5 only); *Statistically significant difference between Group-II and III (only for Days 7 and 10); *From Day 7 onward, only Group-II and III were compared using independent t-test



[Table/Fig-6]: Line graph showing the Endothelial Cell Count (ECC) decline over time stored in cornisol media in various age groups with error bars.

pairwise differences between group I and II, group I and III, and group II and III at appropriate time points based on statistical assumptions. The early and sustained ECC decline in group II and III suggests that endothelial deterioration accelerates beyond day 5 in older donor tissues.

Changes in Hexagonality (%HEX)

Hexagonality declined progressively across all groups. Group I retained near-physiological morphology (from 59.3% to 55.2%), with intragroup change significant (p-value=0.007). Group II showed a moderate decline (p-value <0.001), while group III dropped from 43.1% to 32.1% by day 14 (p-value <0.001), reflecting increasing cellular pleomorphism [Table/Fig-7,8]. The steep morphometric shift in group III suggests cytoskeletal instability or metabolic stress affecting cell regularity.

Changes in Coefficient of Variation (CV%)

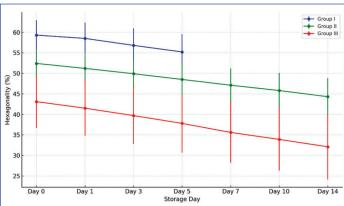
The CV% increased minimally in Group I (28.1% to 31.5%, p-value=0.006), moderately in group II (p-value <0.001), and sharply in group III (61.2% to 73.8%, p-value <0.001) [Tables/Fig-9,10]. These findings confirm that age and prolonged storage lead to increased polymegathism, with group III showing the most pronounced variability in cell size. One-way ANOVA showed statistically significant intergroup differences in CV% across all time points (p-value <0.001). Group III consistently demonstrated higher values than the other two groups, confirming age-related endothelial instability.

Percentage endothelial cell loss over time [Table/Fig-11,12]: Group I showed minimal cell loss (2.1% to 7.8% up to day 5), while groups II and III exhibited steeper declines. Intragroup changes were significant in all three groups (group I: p-value=0.009; Groups II and III: p-value <0.001). One-way ANOVA demonstrated statistically significant intergroup differences in percentage ECC loss at day 3 (p-value <0.001), day 5 (p-value <0.001), and day 14 (p-value=0.041). Intergroup differences were not significant at day 1 (p-value=0.09), day 7 (p-value=0.087), and day 10 (p-value=0.061). Tukey's post-hoc test indicated that group I differed significantly from groups II and III beyond day 3. Day 5 may represent a critical threshold beyond which older donor tissues experience accelerated endothelial loss, emphasising the importance of using such tissues earlier.

ECC variation by cause of death [Tables/Fig-13,14]: ECC decline was steepest in tissues from trauma-related deaths (45.3%), followed by cardiorespiratory failure (41.6%), and was lowest in non traumatic Intracerebral Haemorrhage (ICH) (22.4%). Intragroup differences were significant for all three categories (p-value <0.001). One-way ANOVA revealed significant intergroup differences across

Age group (years)	Hex Day 0 (%) Mean±SD	Hex Day 1 (%) Mean±SD	Hex Day 3 (%) Mean±SD	Hex Day 5 (%) Mean±SD	Hex Day 7 (%) Mean±SD	Hex Day 10 (%) Mean±SD	Hex Day 14 (%) Mean±SD	Mean change±SD	Intragroup p-value
0-30	59.3±3.6	58.5±3.8	56.8±4.1	55.2±4.3	-	-	-	-4.1±2.7	0.007
31-60	52.4±3.2 [†]	51.2±3.4 [†]	49.9±3.6 [†]	48.5±3.9 [†]	47.1±4.1#	45.8±4.3#	44.3±4.5#	-8.1±3.3	<0.001
61-90	43.1±6.4*	41.5±6.7*	39.7±6.9*	37.8±7.1*	35.6±7.4	33.9±7.6	32.1±7.9	-11.0±5.2	<0.001
Intergroup p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.0012	<0.001		

[Table/Fig-7]: Table showing hexagonality (%) of the endothelial cells of the donor tissue over time.
*Significant between Group-I vs III - only where both groups have data (i.e., Day 0-5 only); †Significant between Group-I vs III - only valid Day 0-5; *Significant between Group-II vs III - valid Day 7-14



[Table/Fig-8]: Line graph showing hexagonality of the endothelial cells of the donor tissue over time as compared to the donor age in various age groups with error bars.

all time points except day 5 (p-value=0.071). Pairwise comparisons using Tukey's post-hoc test confirmed significance between trauma-related and non traumatic deaths, between trauma-related and cardiorespiratory failure donors and between cardiorespiratory failure and non traumatic deaths at multiple time points. Trauma-associated endothelial loss suggests that systemic stress at death may compromise corneal integrity, underscoring the need for cautious donor selection.

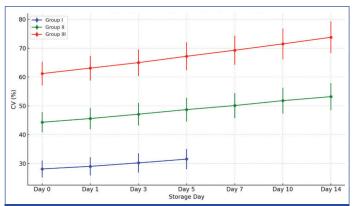
DISCUSSION

The preservation of corneal endothelial parameters is critical for successful corneal transplantation, and this study reaffirms the substantial influence of donor age and storage duration on these parameters. Present study findings show that ECC, cell morphology (hexagonality) and CV are better preserved in younger donor

Age group (in years)	CV Day 0 (%) Mean±SD	CV Day 1 (%) Mean±SD	CV Day 3 (%) Mean±SD	CV Day 5 (%) Mean±SD	CV Day 7 (%) Mean±SD	CV Day 10 (%) Mean±SD	CV Day 14 (%) Mean±SD	Mean CV change±SD	Intragroup p-value
0-30	28.1±2.9	29.0±3.1	30.2±3.3	31.5±3.5	-	-	-	+3.4±2.1	0.006
31-60	44.3±3.5†	45.6±3.7†	47.1±3.9 [†]	48.7±4.1†	50.1±4.3	51.8±4.5	53.2±4.7	+8.9±3.9	<0.001
61-90	61.2±4.1*	63.1±4.3*	65.0±4.6*	67.2±4.8*	69.3±5.0	71.5±5.3	73.8±5.5	+12.6±5.3	<0.001
Intergroup p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

[Table/Fig-9]: Table showing co-efficient of variation (CV)(%) of the endothelial cells of the donor tissues over time.

*p<0.05 between Group-I and Group-III; †p<0.05 between Group-I and Group-II; Statistical significance determined using One-way ANOVA from Day 0 to Day 5; No comparisons involving Group-I were made after Day 5 due to absence of data; From Day 7 to Day 14, only independent t-tests were considered between Group-II and Group-III.



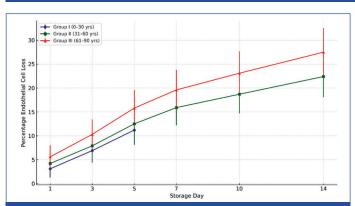
[Table/Fig-10]: Line graph showing co-efficient of variation of the endothelial cells of the donor tissues over time in various age groups with error bars.

In this study, group I (0-30 years) demonstrated the highest baseline ECC and the slowest rate of decline, with ECC loss limited to 7.8% by day 5. In contrast, group II (31-60 years) showed the highest percentage ECC loss, 36.5% by day 14, followed by group III (61-90 years) at 30.4%. These differences were statistically significant across almost all time points (p-value <0.001), with the exception of day 14 (p-value=0.2206). This temporal pattern supports earlier observations by Patel SV et al., and Li JY and Mannis MJ who highlighted that ageing endothelium becomes increasingly vulnerable to storage-induced stress, especially after the first week [6,13].

The decline in hexagonality (%HEX), a key marker of endothelial cell regularity, was also most marked in group III (from 43.1% to 32.1%), while group I maintained hexagonality above 55% during the early storage period. These findings echo reports by Bourne WM and

Age group (in years)	Day 1 (%) Mean±SD	Day 3 (%) Mean±SD	Day 5 (%) Mean±SD	Day 7 (%) Mean±SD	Day 10 (%) Mean±SD	Day 14 (%) Mean±SD	Intragroup p- value
0-30	2.1±1.4	4.6±1.9	7.8±2.4	-	-	-	0.009
31-60	3.2±1.8	6.5±2.3 [†]	10.5±2.8 [†]	14.7±3.3	17.6±3.8	36.5±4.7	<0.001
61-90	4.1±1.7	8.1±2.7*#	12.6±3.4*#	17.5±3.9	21.6±4.0	30.4±4.2	<0.001
Intergroup p-value	0.09	<0.001	<0.001	0.087	0.061	0.041	-

[Table/Fig-11]: Table showing the percentage decrease in endothelial cells of the donor tissue over time in various age groups. *p<0.05 between Group-I and Group-III; *p<0.05 between Group-II and Group-III and Group-III



[Table/Fig-12]: Line graph showing the percentage decrease in endothelial cells of the donor tissue over time in various age groups with error bars.

Kaufman HE and Yamaguchi T et al., where older donor tissues exhibited increased cellular pleomorphism under identical storage conditions [1,14]. The intergroup differences in hexagonality were statistically significant across all time points, further affirming the vulnerability of aged tissues to morphologic alteration.

The CV, which reflects polymegathism or variation in cell size, rose sharply in group III (61.2% to 73.8%) and more modestly in group I (28.1% to 31.5%). Intergroup comparisons across all days revealed statistically significant differences (p-value <0.001). The findings suggest that endothelial stress responses during storage, possibly driven by oxidative and metabolic strain, are amplified in older tissues, which may have limited repair and regulatory capacity [13].

Of particular note, this study included an intergroup comparison of percentage endothelial cell loss [Table/Fig-11]. Intergroup differences

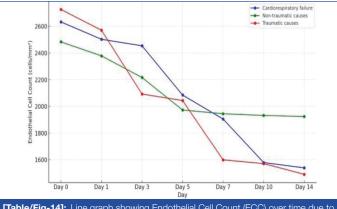
Cause of death of donor	Day 0 Mean±SD	Day 1 Mean±SD	Day 3 Mean±SD	Day 5 Mean±SD	Day 7 Mean±SD	Day 10 Mean±SD	Day 14 Mean±SD	Mean% loss±SD	Intra- group p-value
Cardiorespiratory failure	2633.09±143.2	2503.05±136.4	2454.07±130.6	2085.0±128.7	1905.0±118.7	1579.0±110.3	1539.0±106.9	41.6±5.3%	<0.001
Non-traumatic with ICH	2483.7±152.2 ^{#†}	2378.6±122.2#†	2216.4±143.1#†	1972.1±132.1	1945.0±126.6#†	1932.0±125.6#†	1924.0±123.8 ^{#†}	22.4±3.8%	<0.001
Traumatic ICH	2726.4±158.3*	2570.6±115.3*	2092.4±149.3*	2043.8±142.8	1599.1±138.9*	1571.0±135.7 *	1491.0±132.6 *	45.3±6.1%	<0.001
Intergroup p-value	0.017	0.001	0.001	0.071	<0.001	<0.001	<0.001		

[Table/Fig-13]: Table showing correlation between the cause of death of the donor and the number of endothelial cell loss following the day of excision.

*p<0.05 between Cardiorespiratory failure and Traumatic ICH; *p<0.05 between Non-traumatic ICH and Traumatic ICH; *p<0.05 between Cardiorespiratory failure and Non-traumatic ICH; All intergroup comparisons were performed using one way ANOVA with Tukey's post-hoc test

tissues. This aligns with prior studies that have demonstrated that corneas from younger donors exhibit superior structural integrity and metabolic viability during storage compared with those from older donors [6,8].

were not statistically significant at day 1 (p-value=0.09), but became significant by day 3 (p-value <0.001) and remained significant at day 5 (p-value <0.001). Significance attenuated at day 7 and day 10 (p-value=0.087 and p-value=0.061, respectively) before reaching



[Table/Fig-14]: Line graph showing Endothelial Cell Count (ECC) over time due to various causes of death.

significance again at day 14 (p-value=0.041). Taken together, these results indicate that divergence between age groups emerges early during storage (by day 3), is most pronounced by day 5, and despite fluctuations at intermediate time points persists at the end of the two-week storage period. This suggests that endothelial deterioration in older donor tissue accelerates after day 5. Day 5 may represent a critical window beyond which the viability of endothelial cells in older donor groups deteriorates rapidly. This is clinically relevant, as it supports prioritising early utilisation of corneas from older donors to minimise post-transplant cell loss [6,8].

The present study also explored the relationship between cause of donor death and endothelial viability [Table/Fig-13]. Donor tissues from trauma-related deaths exhibited the greatest ECC loss (45.3%), followed by cardiorespiratory failure (41.6%) and non traumatic intracranial haemorrhage (22.4%). These differences were statistically significant across almost all storage time points (except Day 5 where ANOVA was non significant) and pairwise comparisons confirmed meaningful intergroup separation. These findings concord with those by Angunawela RI et al., and Mannis MJ et al., who suggested that systemic metabolic stress and multiorgan failure in traumatic and cardiac deaths may contribute to endothelial compromise, even in the absence of overt ocular trauma [15,16].

In summary, this study demonstrates that donor age and cause of death significantly influence corneal endothelial survival during Cornisol storage. The inclusion of both intragroup and intergroup analyses, as well as time-specific pairwise comparisons, strengthens the robustness of these findings and supports practical recommendations for corneal tissue utilisation.

Limitation(s)

This study was limited by its sample size within group I and group III, which may restrict the generalisability of subgroup comparisons. Data for group I were available only up to day 5, precluding intergroup comparisons among all three groups beyond this point and preventing longer-term analysis in younger donor tissues. Additionally, while the cause of death was recorded, systemic co-

morbidities and the duration of hospitalisation prior to death were not accounted for, both of which may influence endothelial health. Finally, although Cornisol is widely used in India, results may not be directly extrapolatable to other storage media. Further multicentre studies with larger and more balanced sample sizes across all age groups, along with post-transplant outcome correlation, are recommended.

CONCLUSION(S)

This study highlights that donor age, storage duration and cause of death significantly affect corneal endothelial viability. Younger donors exhibited better preservation of ECC, hexagonality and CV during Cornisol storage. Endothelial deterioration was most notable in older donor groups and after day 5 of storage, with significant intergroup differences observed across most time points. Additionally, tissues from trauma-related deaths showed the highest cell loss, followed by cardiorespiratory failure and non traumatic intracranial haemorrhage. These findings support early utilisation of older donor corneas and careful donor selection to optimise transplant outcomes.

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PARTICULARS OF CONTRIBUTORS:

- 1. Professor and Head, Department of Ophthalmology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India.
- 2. Resident, Department of Ophthalmology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India.
- 3. Cornea Consultant, Department of Ophthalmology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India.
- 4. Resident, Department of Ophthalmology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Pradipta Potdar,

B/1703, Mahavir Universe LBS Marg Bhandup West, Mumbai-400078, Maharashtra, India.

E-mail: Pradi.potdar@gmail.com

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jun 29, 2025
- Manual Googling: Jul 14, 2025
- iThenticate Software: Aug 25, 2025 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 9

Date of Submission: Jun 14, 2025 Date of Peer Review: Jul 05, 2025 Date of Acceptance: Aug 27, 2025 Date of Publishing: Oct 01, 2025