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# EVALUATION OF ENDOTHELIAL CELL COUNT OF CORNEAL GRAFT AFTER PENETRATING KERATOPLASTY

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## ORIGINAL ARTICLE

# **Evaluation of Endothelial Cell Count of Corneal Graft After Penetrating Keratoplasty**

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#### Abstract

*Background*: A surgical procedure known as penetrating keratoplasty (PKP) involves replacing donor tissue throughout the entire recipient corneal thickness. Good to exceptional quality donor corneal tissues are preferred by PKP surgeons because they give enough endothelial cells for a lifetime.

*Objective*: The aim of the study was to assess endothelial cell count one and half month after penetrating keratoplasty (PKP) by using specular microscopy.

*Method*: It is cross-sectional, descriptive Study which done at Giza memorial institute of ophthalmic research from September 2021 to June 2022. This study WAS performed on 30 eyes of 30 patients that underwent penetrating keratoplasty.

*Results*: Comparison of endothelial cell loss between patients according to indication showed that the loss was statistically significant higher in opacity group than KC group, where endothelial cell loss ranged from 512 to 799 cell/mm<sup>2</sup>, mean  $\pm$  SD. Was 636.8  $\pm$  79.28, and median 641 in KC group, and ranged from 502 to 1011 cell/mm<sup>2</sup>, mean  $\pm$  SD. Was 759.5  $\pm$  149.0, and median 715 in opacity group.

*Conclusion*: Our research showed that following PKP, the loss of endothelial cells is greatly accelerated. We also noticed that a number of factors affect how quickly endothelial cells are lost following PKP, where the recipient age and the death to preservation time were positively associated with ECD loss, and higher in opacity group than KC group and not influenced by donor age and preservation to surgery time.

Keywords: Corneal graft, Endothelial cell count, Evaluation, Penetrating keratoplasty

#### 1. Introduction

A surgical procedure known as penetrating keratoplasty (PKP) involves replacing donor tissue throughout the entire recipient corneal thickness. Good to exceptional quality donor corneal tissues are preferred by PKP surgeons because they give enough endothelial cells for a lifetime.

Corneal graft survival depends on many factors such as: donor age, endothelial cell density, death to preservation time and death to surgery time.

#### 1.1. Indications of keratoplasty

The four categories of indications for corneal transplantation are optical (to restore vision),

tectonic/reconstructive (to restore the globe's anatomy), and therapeutic (to remove corneal tissue which is infected or inflamed and unresponsive to treatment). The most common procedures are tectonic and therapeutic keratoplasties, then optical keratoplasties.<sup>1</sup> Full-thickness penetrating keratoplasty and partial lamellar corneal operations, such as anterior lamellar and posterior lamellar keratoplasty, are two categories for corneal transplantation treatments.<sup>2</sup>

The cornea's innermost layer, the corneal endothelium, is made up of just one layer of cuboidal cells. These cells play a crucial part in preserving the cornea's transparency and moisture.<sup>3</sup>

With ageing, trauma, inflammation, surgery, and disease processes like Fuchs' endothelial dystrophy,

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the number of endothelial cells diminishes. Endothelial cell density is roughly 3500-4000 cells/mm<sup>2</sup> at birth, progressively declining to 2500 cells/mm<sup>2</sup> at age 50 and 2000 cells/mm<sup>2</sup> at age 80, at an average annual rate of 0.6%.<sup>4</sup>

The aim of the study was to assess endothelial cell count one and half month after penetrating keratoplasty (PKP) by using specular microscopy.

#### 2. Patients and methods

It is cross-sectional, descriptive Study. Giza memorial institute of ophthalmic research from september, 2021 to June 2022. This study WAS performed on 30 eyes of 30 patients that underwent penetrating keratoplasty.

#### 2.1. Inclusion criteria

Donor corneas obtained from the Eye Banks under international eye bank standards: Minimal endothelial cellular density 2000/mm<sup>2</sup>, death to preservation time less than 24 h and estimated time from donor death to surgery not longer than 14 days. Any indication for PKP e.g., restores sight, infected or inflamed corneal tissue, corneal decompensation, opacities, trauma, etc.

#### 2.2. Exclusions criteria

Previous attacks of graft rejection, additional intraocular surgery before, during or after transplantation, e.g., cataract, extraocular surgeries and trabeculectomy, associated ocular pathology, e.g., glaucoma, uveitis and extraocular diseases, collagen vascular diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus. Stevens-Johnson syndrome, Sjogren's disease, and other ocular surface issues may make it difficult to do good specular microscopy.

#### 2.3. Methods

Data obtained from our records included: Preoperative diagnosis (indication of penetrating keratoplasty), recipient age and Sex, donor age and Sex, death to preservation duration, preservation time (Duration between Preservation and Operation) and graft endothelial cell density.

#### 2.4. Preoperative assessment

A thorough medical and ocular history was collected. Emphasis was placed on the patient's profession, eye trauma history, and any prior ocular surgery.

#### 2.5. Preoperative preparation

To protect the lens during surgery, the pupil was constricted pre-operatively with 1% pilocarpine.

#### 2.6. Anesthesia

Except for younger patients, all patients were operated on under local anaesthetic.

#### 2.7. Preparation of donor cornea

A sharp, disposable trephine was held perpendicular to the donor cornea and trephined with the endothelial side facing up. The host trephine was 0.5 mm smaller than the donor tissue trephine.

#### 2.8. Preparation of the recipient bed

The area in question was measured using a calliper. Using a trephine at least 1 mm larger than the lesion's size increased the likelihood of a stable recipient bed free from infection. Lance tip blade was used to make a guarded entrance into the anterior chamber, and then corneoscleral extension scissors were used to remove the infected cornea.

# 2.9. Placement of the donor corneal tissue in the host bed

The tissue was transferred to the recipient bed and placed on viscoelastic material after being delicately gripped with fine-toothed forceps at the junction of the epithelium and stroma.

#### 2.10. Placement of four interrupted radial 10-0 nylon cardinal sutures

#### 2.10.1. Investigations

All postoperative specular microscopy Measurements of the corneal endothelium were acquired via the specular microscopy (SP-1P, TOPCPN, Japan), after one and half month from the surgery.

#### 2.10.2. Ethical consideration

After being told about the study, each participant's written agreement was obtained. The study follows the 1964 Helsinki Declaration and its later amendments, as well as the Al-Azhar Medical Research Ethics Committee's code of ethics.

#### 2.11. Statistical analysis of the data

Data were entered into the computer and assessed using the IBM SPSS software package, version 20.0.Apple Inc., Armonk, New York Number and percentage were used to describe qualitative data. The normality of the distribution was examined using the Shapiro–Wilk test. The range (minimum and maximum), mean, standard deviation, median, and interquartile range were used to characterise quantitative data (IQR). At the 5% level, significance of the results was determined. The tests used were: Student t-test: To compare two groups under study for quantitative variables with normally distributed distributions. Correlation between two quantitative variables that are normally distributed using the Pearson coefficient.

#### 3. Results

#### Table 1.

This table shows the distribution of age and Sex in both recipient and donors for penetrating keratoplasty, where the recipients were 10 (33.3%) males, and 20 (66.7%) females, and their age ranged from 23 to 63 years, mean  $\pm$  SD. was 37.37  $\pm$  11.07, and median (IQR) 34.50 (29.0–46.0), and the donors were 18 (60%) males, and 12 (40%) females, and their age ranged from 29 to 76 years, mean  $\pm$  SD. was 60.30  $\pm$  10.9, and median (IQR) 62.50 (57.0–66.0) (Table 2).

Endothelial cell density graft count (preoperative) ranged from 2024 to 3367 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 2442.23  $\pm$  372.22, and median (IQR) 2315 (2179–2681), and the endothelial cell density spec count (1.5 month postoperative) ranged from 1502 to 2649 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 1764.57  $\pm$  308.73, and median 1625.50 (1562–1882), the reduction in endothelial cell density mean  $\pm$  SD. was 677.67  $\pm$  120.30, and the reduction percentage was 27.85  $\pm$  3.49% (Table 3).

Death to preservation duration ranged from 0.35 to 22.7 h, mean  $\pm$  SD. was 9.36  $\pm$  6.08, and median (IQR) 9.23 (4.87–12.02), and the Preservation to surgery time ranged from 5 to 11 days, mean  $\pm$  SD. was 7.83  $\pm$  1.72, and median 7.50 (7.0–9.0) (Table 4).

There was positive correlation between endothelial cell loss and age of recipient (years) where

Table 1. Distribution of the studied cases according to demographic data (n = 30).

Demographic data	Recipient number (%)	Donor number (%)	
Sex			
Male	10 (33.3)	18 (60.0)	
Female	20 (66.7)	12 (40.0)	
Age (years)			
Min. – Max.	23.0-63.0	29.0-76.0	
Mean $\pm$ SD.	$37.37 \pm 11.07$	$60.30 \pm 10.91$	
Median (IQR)	34.50 (29.0-46.0)	62.50 (57.0-66.0)	

IQR, Inter quartile range; SD, Standard deviation.

Table 2. Descriptive analysis of the studied cases according to endothelial cell density (n = 30).

Endothelial cell	Graft count	Spec count
density (cell/mm <sup>2</sup> )	(Preoperative)	(1.5 month postoperative)
Min. – Max. Mean ± SD. Median (IQR) Reduction % of Reduction	$\begin{array}{c} 2024-3367\\ 2442.23 \pm 372.22\\ 2315 \ (2179-2681)\\ 677.67 \pm 120.30\\ 27.85 \pm 3.49 \end{array}$	$\begin{array}{c} 1502-2649\\ 1764.57 \pm 308.73\\ 1625.50 \ (1562-1882) \end{array}$

IQR, Inter quartile range; SD, Standard deviation.

Table 3. Descriptive analysis of the studied cases according to death to preservation duration and preservation to surgery time (n = 30).

	Min. – Max.	Mean $\pm$ SD.	Median (IQR)
Death to preservation duration (hours)	0.35-22.70	9.36 ± 6.08	9.23 (4.87–12.02)
Preservation to surgery time (days)	5.0-11.0	7.83 ± 1.72	7.50 (7.0–9.0)

IQR, Inter quartile range; SD, Standard deviation. **Preservation time:** Duration between Preservation and Operation.

Table 4. Correlation between endothelial cell loss and different parameters (n = 30).

Endothelial cell loss vs.	r	Р
Age of recipient (years)	0.381	0.038*
Age of donor (years)	0.189	0.316
Death to preservation duration (hours)	0.474	0.008*
Preservation to surgery time (days)	0.275	0.141

r, Pearson coefficient.

\*Statistically significant at  $P \leq 0.05$ .

(r = 0.381, P = 0.038), and death to preservation duration (hours) where (r = 0.474, P = 0.008), and there was no correlation between endothelial cell loss and Age of donor (years), and preservation to surgery time (days) (Table 5).

Penetrating keratoplasty indication was KC in 20 (66.7%), and opacity in 10 (33.3%) (Table 6).

Comparison of endothelial cell loss between patients according to indication showed that the loss was statistically significantly higher in the opacity group than the KC group, where endothelial cell loss ranged from 512 to 799 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 636.8  $\pm$  79.28, and median 641 in the KC group, and ranged from 502 to 1011 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 759.5  $\pm$  149.0, and median 715 in opacity group.

Table 5. Distribution of the studied cases according to indication (n = 30).

Indication	Number (%	
КС	20 (66.7)	
Opacity	10 (33.3)	

KC, keratoconus.

Table 6. Relation between indication and endothelial cell loss (n = 30).

	Indication		t	Р
	KC ( <i>n</i> = 20)	Opacity ( $n = 10$ )		
Endothelial cell loss				
Min. – Max.	512.0-799.0	502.0-1011.0	2.438*	0.032*
Mean $\pm$ SD.	$636.8 \pm 79.28$	$759.5 \pm 149.0$		
Median	641.0	751.0		

SD, Standard deviation; t, Student t-test.

p: P value for comparing between KC and opacity.

\*Statistically significant at  $P \leq 0.05$ .

#### 4. Discussion

The global population of blind people is estimated to be 50 million. Additionally, 'low eyesight' affects about 150 million people. There are 200 million severely visually impaired people in the world today, which is unacceptable situation from a social and economic standpoint. Blindness prevalence is also expected to rise by almost 2 million people annually as a result of the population's ageing and the slow pace of service provision.<sup>5</sup>

Our results showed the age and Sex distribution in penetrating keratoplasty donors and recipients. The grantees' ages ranged from 23 to 63, and there were 10 (33.3%) men and 20 (66.7%) women among them. The median (IQR) was 34.50, while the mean SD was 37.37  $\pm$  11.07. (29.0–46.0). The donors' ages ranged from 18 (60%) males to 12 (40%) females (57.0–66.0). Based on Inoue et al.,<sup>6</sup> analysis of changes in corneal endothelial cells 20 years after penetrating keratoplasty, the average age of these donors was 65.3  $\pm$  14.4 years (range, 41–82 years).

The patients' ages ranged from 6 to 45 years, with a mean of  $25.3 \pm 10.4$  years, at the time of operation. When endothelial cell density and corneal thickness were measured in corneal grafts an average of 5 years following penetrating keratoplasty, Kettesy et al.<sup>7</sup> discovered that the mean age of recipient patients was  $42.4 \pm 17.1$  years at the time of the examination. The average age of the donors was 66.2 years old. 149 men (65.9%) and 77 women (34.1%), totaling 226 instances, were found when Xu et al.<sup>6</sup> looked into the risk factors for the early phases of rapid graft endothelium attenuation following penetrating keratoplasty. The patients were 59 years old on average (range, 15-88 years). The median follow-up time was 20 months (range, 12-35 months).

In our study endothelial cell density graft count (preoperative) ranged from 2024 to 3367 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 2442.23  $\pm$  372.22, and median (IQR) 2315 (2179–2681), and the endothelial cell density spec count (1.5 month postoperative) ranged from 1502 to 2649 cell/mm<sup>2</sup>, mean  $\pm$  SD. was

1764.57  $\pm$  308.73, and median 1625.50 (1562–1882), the reduction in endothelial cell density mean  $\pm$  SD. was 677.67  $\pm$  120.30, and the reduction percentage was 27.85  $\pm$  3.49%.

Xu, al<sup>6</sup> showed that Infectious keratopathy (127 cases, 56.2%) was the primary cause of penetrating keratoplasty, with fungal keratitis accounting for the majority of cases (61 cases, 27.0%), followed by herpes simplex keratitis (44 cases, 19.5%), in the analysis of risk factors for rapid attenuation of graft endothelium in the early stage after penetrating keratoplasty. Non-infectious keratopathy was most commonly caused by corneal endothelial decompensation (47 cases, 20.8%), followed by keratoconus and corneal leukoplakia (both 17 instances, 7.5%). The donors' average age was 57.3  $\pm$  14.2 years (range, 18-90 years). 1.5 h was the average amount of time between death and preservation (range, 0-16 h). The average number of days between death and operation was three (range, 0-9 days).

Penetrating keratoplasty indication was Keratoconus in 20 (66.7%), and opacity in 10 (33.3%). Comparison of epithelium cell loss between patients in line with indication showed that the loss was statistically vital higher in opacity cluster than rate cluster, wherever epithelium cell loss ranged from 512 to 799 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 636.8  $\pm$  79.28, and median 641 in rate cluster, and ranged from 502 to 1011 cell/mm<sup>2</sup>, mean  $\pm$  SD. was 759.5  $\pm$  149.0, and median 715 in opacity cluster.

According to Kettesy et al.<sup>7</sup> studied the assessment of endothelial cell density and corneal thickness in corneal grafts an average of 5 years after penetrating keratoplasty, and found that Causative diagnosis was keratoconus (n = 36), bullous keratopathy (n = 14), corneal leucoma (n = 9), herpetic keratitis (n = 7), acanthamoeba keratitis (n = 1) or corneal dystrophy (n = 1). At examination time, the overall ECD was 1501 ± 249 cell/mm<sup>2</sup> (ranging from 1100 to 2225 cell/mm<sup>2</sup>).

Patient age and the way abundant time from death to safeguarding, in any case, did not vary essentially between the 2 gatherings. At one year postoperatively, 76 (33.6%) eyes had ECD 1000/mm<sup>2</sup>, and also the patients during this gathering were unnoticeably a lot of seasoned (P = zero.024). At surgical year, 51 (22.6%) eyes had ECD >2000/mm<sup>2</sup>, and also the age of the givers during this gathering was essentially lower (P = zero.016).<sup>6</sup>

A gamble issue for associate degree ECL pace of over half-hour at surgical one month was a ending to protection season of >60 min Albeit the issue that matters was not measurably large, the extent of patients with high epithelium misfortune rates at surgical multi month and one year was higher at death to process time > three days than at DTT three days. ECD one thousand cells/mm<sup>2</sup> was primarily as high as fifty one.1%, that was essentially more than that in several gatherings (29.1%, P = 0.004), whereas the amount of ECD >2000 cells/ mm<sup>2</sup> was simply eight.5%, that was altogether lower in several gatherings (26.3%, P = 0.01) in patients matured > sixty years, WHO were a big gamble issue for ECL rate > half-hour at one surgical month.<sup>6</sup> Patients with irresistible keratopathy had a lower predominance of ECL than patients with nonkeratopathy, and epithelium irresistible cell misfortune was unaffected by understanding sex, elementary illness, or means of life choices like smoking and drinking.<sup>6</sup>

#### 5. Conclusion

Our research showed that endothelial cell death is significantly accelerated following PKP. We also observed that the rate of endothelial cell loss after PKP is influenced by several factors, where the recipient age and the death to preservation time were positively associated with ECD loss, and higher in opacity group than KC group and not influenced by donor age and preservation to surgery time.

#### Authorship

All authors have a substantial contribution to the article.

#### Disclosure

The authors have no financial interest to declare in relation to the content of this article.

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#### **Conflict of Interest**

The authors declared that there were no conflicts of interest.

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