

## Analysis of the cornea donor data: an eye bank study

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### ABSTRACT

**Objectives.** To analyse 1.5-year data of our newly established eye bank and to evaluate the factors affecting donor quality. **Methods.** Our bank's donor cornea data between July 2013 and November 2014 were retrospectively analysed. The effects of donor age, sex, and time from death to harvesting on the findings of specular microscopy were assessed. **Results.** A total of 139 corneas retrieved from 70 donors. The mean age of donors was  $34.2 \pm 14.6$  (5-64) years. The mean time from death to harvesting was  $6.7 \pm 2.9$  (1-13) hours; the mean time from collection to transplantation was  $5.2 \pm 2.8$  (1-14) days. Age had a significant negative correlation with mean endothelial cell count (ECC), a significant positive correlation between mean cell area (MCA) and standard deviation (SD). Time from death to harvesting had a significant negative correlation with cell count and 6A; it had a significant positive correlation with SD, the coefficient of variation, and MCA. **Conclusion.** According to the results of the present study, ECC, MCA, and SD levels were greater in younger donors. Endothelial morphology was altered as the time from death to harvesting was prolonged; however, the alteration in cell morphology was not severe enough to alter transplantation success with the corneas being harvested within the first 13 hours.

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**Keywords:** Eye Bank; cornea; specular microscopy; endothelial; donor

### Introduction

Eye banks are special medical units responsible for harvesting donor corneal tissue required for the cornea transplantation from suitable donors, preserving harvested tissues under appropriate conditions, evaluating tissue quality for transplantation, transporting tissues to transplant centres under optimal conditions, registering patients who apply for keratoplasty procedure, as well as establishing and maintaining communication with the

patients. Donor suitability, time from donor death to tissue harvesting, cornea storage conditions, and time of harvesting to transplantation are vital factors for graft survival during the time of harvesting to transplanting corneal tissue. Corneal storage duration is dependent on corneal preservation methods (tissue culture medium, organ culture, moist chamber); corneal tissue should be transplanted as soon as possible before irreversible degeneration occurs after

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donor death and during storage [1].

As a result of continuously increasing the number of patients entering keratoplasty waiting lists and prolonged waiting durations, Turkish Ministry of Health has recently funded the establishment of new eye banks in various cities. As of the time of the writing this paper, there are 28 functioning eye banks in Turkey. Our eye bank was established in July 2013 by ministerial approval of Turkish Ministry of Health and supplied 139 donor corneal tissues between July 2013 and November 2014. In the following we want to assess the first donor data of our newly established eye bank.

## Methods

Data of donor corneas harvested at Bursa Yuksek Ihtisas Training and Research Hospital Eye Bank between July 2013 and November 2014 were retrospectively reviewed. Data on donor age, sex, time of death to harvesting, time from collection to transplantation, serology results, specular microscopy (SM) results, and donor usage were recorded. In specular microscopy, the Centre Method was used to determine the corneal endothelial count and endothelial structure (polymegathism and pleomorphism) (Konan Eye Bank Kerato Analyzer, Konan Medical Inc., Japan). The effects of donor age, sex, and time from death to cornea harvesting on the findings of specular microscopy were analysed. The local ethics committee approved the study. After being harvested from the cadavers using the sclerocorneal button technique, donor corneas were preserved in the preservation solution (Eusol C, Alchimia, Italy) at 4°C; and endothelial photographs were taken to be examined for four times from all corneas by the same person using specular microscopy. For each donor cornea, endothelial cell count (ECC), mean cellular area (MCA), standard deviation (SD), hexagonality ratio (6A), and coefficient of variance (CV) were recorded. The donors were categorized into four age groups. Donor gender distribution, corneal side distribution, endothelial count, SD, CV, 6A, MCC, time from death to harvesting and time from collection to transplantation were compared across the four age groups.

### Statistical analysis

Descriptive statistics included mean, standard

deviation, median, minimum, maximum, frequency, and percentage. The distribution of study data was tested with the Kolmogorov-Smirnov test. Quantitative data were analysed with ANOVA and Kruskal-Wallis (Mann-Whitney U) tests. Chi-Square test was used for the analysis of qualitative data. SPSS 22.0 software package was used for all statistical comparisons.

## Results

Among 70 donors included in the study, 16 (23%) were female, and 54 (77%) were male. During our study period, 139 corneal tissues were retrieved from 70 donors. A pair of corneas was harvested from 69 donors and a single cornea from 1 donor. The mean age of the donors was 34.2±14.6 (5-64) years. The mean time from death to harvesting was 6.7±2.9 (1-13) hours; the mean time from collection to transplantation at different centres was 5.2±2.8 (1-14) days. The mean age of the transplant recipients was 45.8±25 (1-88) years at various centres. The obtained corneas were serologically tested using anti-HIV, HBsAg, anti-HCV and VDRL tests. Two corneas were HBsAg positive and were thus disposed of.

Another cornea was disposed of by a recipient centre due to suspected infection. The findings of specular microscopy, age and sex group distribution of corneal tissues were shown in Table 1.

There were no significant differences between the age groups on gender distribution and the distribution of corneal tissue side. The cell count was significantly greater in the first age group (5-19 years) compared with the second (20-34 years), third (35-49 years), and fourth (50-65 years) age groups ( $p<0.001$ ). The cell count was significantly greater in the second age group compared to the 3<sup>rd</sup> and 4<sup>th</sup> age groups ( $p<0.001$ ) (Table 2). The SD level was significantly lower in the first age group compared to the second, third, and fourth age groups ( $p<0.001$ ). It was also significantly lower in the second age group compared to the fourth age group ( $p<0.001$ ) (Table 2). There were no significant differences between the age groups on CV, 6A, time from death to harvesting, and time from collection to transplantation (Table 2). The first age group had a significantly lower MCA compared to the second, third, and fourth age groups ( $p<0.001$ ) The MCA level was significantly lower in the second age group compared to the 3<sup>rd</sup> and 4<sup>th</sup> age groups ( $p<0.001$ )

**Table 1.** Specular microscopy, age and sex group distribution of corneal tissues

	<b>Min-Max</b>	<b>Median</b>	<b>Mean ± SD</b>
<b>Donor Age</b>	5-64	34	34.2 ± 14.6
<b>Age Groups</b>	5-19		34 (24.5%)
	20-34		37 (26.6%)
	35-49		43 (30.9%)
	50-65		25 (18.0%)
<b>Cell Count</b>	2008 – 3968	2857	2884.0 ± 457.3
<b>SD</b>	53.0 – 257.0	106.0	112.7 ± 36.2
<b>CV</b>	19.0 – 60.0	30.0	31.4 ± 6.7
<b>6A</b>	39.0 – 81.0	61.0	61.1 ± 8.6
<b>MCA</b>	252.0 – 498.0	347.0	354.3 ± 58.4

SD= Standart deviation, CV=coefficient of variance, 6A=hexagonality ratio, MCA=mean cellular area

( Table 2).

Cell count, SD, CV, 6A, and MCA levels were not significantly different between both genders (Table1). Time from death to harvesting had a significant negative correlation between cell count and 6A ( $p=0.005$ ). It had a significant positive correlation with SD, CV ( $p<0.001$  for both), and MCA ( $p=0.011$ ) (Table 3). Twenty-nine (21%) of the harvested corneas were used at our hospital while the remaining 108 (79%) corneas were sent to other requesting institutions. Two corneas had HBsAg positivity and were thus disposed of.

## Discussion

Today, there is an estimated 4.9 million patients with bilateral corneal blindness worldwide, especially in the developing countries, who may potentially

regain their visual ability with corneal transplantation. According to the World Health Organisation data, 120.000 keratoplasties (KP) operations were performed in 2000 worldwide [2, 3]. According to the data of the Turkish Ministry of Health, the numbers of keratoplasty operations were 1784 and 1921 in 2012 and 2013, respectively. The official number of patients who are in the cornea waiting lists is 4822, although unofficial estimates put the number at 10.000 [5, 6].

Evaluation of donor candidacy according to certain standards is vital to operational success and preventing complications. For this purpose, the European Eye Bank Association (EEBA) and the Eye Bank Association of America (EBAA) have set up the minimum medical standards for donor candidacy [7, 8]. FDA undertakes supervision and certification of the eye banks in the United States. The available standards are reviewed twice a year, published by the American Academy of Ophthalmology, and

**Table 2.** Age groups with the distribution of specular microscopy findings.

<b>Age groups</b>	<b>5-19 Years</b>	<b>20-34 Years</b>	<b>35-49 Years</b>	<b>50-65 Years</b>	<b>p</b>
<b>Cell Count</b>	3319 ± 361	2947 ± 358*	2685 ± 366*#	2518 ± 326*#	<0.001
<b>SD</b>	95.2 ± 42.6	113.7 ± 31.7*	114.9 ± 26.9*	133.0 ± 35.5*#	<0.001
<b>CV</b>	30.3 ± 8.9	32.7 ± 6.4	30.3 ± 5.0	32.6 ± 5.8	0.051
<b>6A</b>	63.9 ± 10.0	59.2 ± 6.9	59.9 ± 8.2	61.5 ± 8.5	0.111
<b>MCA</b>	304.8 ± 38.5	343.2 ± 41.5*	377.7 ± 52.9*#	403.8 ± 53.8*#	<0.001
<b>Harvesting time (h)</b>	6.1 ± 2.7	6.7 ± 3.2	6.7 ± 2.8	7.3 ± 3.0	0.504
<b>Transplantation time (h)</b>	4.7 ± 3.1	5.7 ± 3.2	4.8 ± 2.5	5.7 ± 2.4	0.212
<b>Recipient Age</b>	32.6 ± 25.6	42.6 ± 23.9*	55.4 ± 21.1*#	52.1 ± 20.7*	0.001

SD= Standart deviation, CV=coefficient of variance, 6A=hexagonality ratio, MCA=mean cellular area, Kruskal-Wallis / ANOVA, \*  $p<0.05$  vs. 5-19 years age group / #  $p<0.05$  vs. 20-34 years age group

**Table 3.** Time from death to harvesting (Spearman Correlation)

		Cell Count	SD	CV	6A	MCA
<b>Time from death to harvesting (Hour)</b>	r	-0.240	0.349	0.394	-0.191	0.224
	p	0.005	0.000	0.000	0.031	0.011

SD= Standart deviation, CV=coefficient of variance, 6A=hexagonality ratio, MCA=mean cellular area,

distributed to eye banks that are members of EBAA [9].

A donor cornea having appropriate criteria for transplantation is one of the most important factors determining the keratoplasty success. Therefore, a harvested cornea should undergo a specular microscopic and serological evaluation before keratoplasty. Some studies using specular microscopy have shown that race, sex, and age of a person may alter morphological properties of the endothelium [10, 11]. It has also been reported that certain factors such as time of donor death to cornea harvesting and cause of donor death may also influence endothelial morphology [12, 13].

Some studies have reported that endothelial count is the most important factor for donor quality. Endothelial cell density is markedly reduced until early puberty, especially in the first couple of years after birth. Former studies have shown that the mean endothelial cell count is reduced, and pleomorphism is markedly increased after the age of 50 [14, 15]. Mean endothelial cell count is reportedly decreased by 0.3% to 0.6% each year while polymegathism and polymorphism simultaneously increase [16, 17]. Endothelial cell density is reduced between the second and eighth decades, dropping from 3000 to 4000 cell/mm<sup>2</sup> on average to as low as 2600 cell/mm<sup>2</sup> on average. The hexagonal cell percentage also drops from 75% to 60% [18], reducing the rate of usage of corneas obtained from elderly donors for transplantation. However, many studies have reported that corneas from advanced-age donors can also be sometimes used for transplantation, and thus donor age does not affect donor survival. Linke *et al.* [17] showed that 32.1% of corneas from donors aged over 80 years met the appropriate criteria for transplantation. Patel *et al.* [19] similarly demonstrated that 80% of corneas from advanced age donors had suitable standards for transplantation. According to EBAA criteria reported in 2006, donors should be between 10 and 75 years of age [20]. Besides, the minimum medical standards set by EBAA in 2013, as well as those set by EBAA in 2012, do not

specify an age limit for donor eligibility [7,8]. Kartal *et al.* [21] reported that ECC showed a significant negative correlation with age, being significantly greater in the first two decades of life. Likewise, our study revealed a negative correlation between ECC and age. Cell count was significantly higher in the first age group (5-19 years) compared with the second (20-34 years), third (35-49 years), and fourth (50-65 years) age groups. Cell count was significantly greater in the second age group compared to the third and fourth age groups (Table 3). Our study also demonstrated a positive correlation between age and MCA, SD values. SD and MCA levels were significantly lower in the first age group compared to the second, third, and fourth age groups. MCA was significantly lower in the second age group compared to the 3rd and 4th age groups, while SD was significantly lower in the second age group than the fourth age group. CV and hexagonality did not show any age-based differences. Some studies in the literature failed to show any age-associated differences in CV, MCA, and hexagonality, although some others have reported age-related differences in MCA [22, 23]. Kartal *et al.* [21] showed that CV and MCA were significantly lower in Group 1 and 2 (0-40 years of age). In our study, mean endothelial count was 2884.0±457.3, mean SD was 112.7±36.2, mean CV was 31.4±6.7, mean MCA was 354.3±58.4 and mean hexagonality was 61.1±8.6. Since our mean donor age was lower than those reported in the literature, specular microscopy findings were ideal for transplantation in all of our donors. Some studies investigated gender-based differences in endothelial cell morphology, some of them reporting ECC differences between both genders, but some others not [24, 25]. Kartal *et al.* [21] reported that female donors had a greater MCA, while a study from India, reported higher CV in female donors [26]. Our results did not reveal any significant difference between both sexes. Previous studies have shown a significant impact of time from donor death to cornea harvesting on endothelial count [17, 27]. EBAA recommends cornea harvesting within 20 hours in those aged less than 50 years, and within 18 hours in

those aged equal to or greater than 50 years of age [20]. As a general rule, it is recommended that corneal harvesting should be completed within 12 hours [28], although there is still no consensus regarding the upper limit of this time, and it may well be prolonged in some cases [29]. Kartal *et al.* [21] showed that ECC was greatest in the  $\leq 3$  hours group, although the difference was statistically non-significant. Our study showed a significant negative correlation between time from death to cornea harvesting and cell count, 6A. There was, however, a significant positive correlation between time from death to cornea harvesting and SD, CV, and MCA.

These results suggest that donor cornea quality is reduced as the time to the collection is increased. However, corneal endothelial structures did not undergo a degenerative process to preclude transplantation since corneas were harvested within maximum 13 hours. The harvested corneas should be serologically tested with anti-HIV, HBsAg, anti-HCV, and VDRL tests. Some domestic prevalence studies at different times have found a mean HBsAg positivity rate of 5% and a mean HCV positivity rate over 0.5% [30].

Kocazeybek *et al.* [31] reported corresponding figures of 6.6% and 0.9%. Our study revealed HBsAg positivity in two corneas, which were disposed of. One other cornea was disposed of by another centre due to suspected infection.

Of the donor corneas collected by our bank over a period of 1.5 years, 21% were used for transplantation by our hospital and 79% by other institutions. Various studies have reported that about 30% of donor corneas cannot be used owing to either serological results or an inappropriate endothelial morphology on specular microscopy examination [8]. We suggest that, we had a higher rate of cornea usage and we generally used high-quality corneas, because our eye bank preferred relatively younger donors, it rejected corneas with suspected infection due to prolonged stay at intensive care unit or the corneas which would be inappropriate for transplantation due their general appearance, and it harvested corneas at  $6.7 \pm 2.9$  hours on average after donor death.

The harvested corneas were sent by our eye bank to other clinics as soon as possible when they were not to be used at our hospital. Time from harvesting to transplantation was  $5.2 \pm 2.8$  (1-14) days on average at other clinics.

In conclusion, we revealed that ECC, MCA, and SD values were greater in younger donors; endothelial

morphology was altered as the time from death to harvesting was prolonged; however, the alteration in cell morphology was not too severe to alter transplant success with the corneas being harvested within first 13 hours. Eye banks should meticulously work in every step from donor harvesting to cornea transplantation. High-risk donor corneas should not be harvested. Harvested corneas should be examined serologically, as well as under specular microscopy, and they should be sent to centres of transplantation as soon as possible.

#### *Conflict of interest*

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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