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Original Research Article

"Specular Microscopy Analysis of Donor Corneal Tissue in a Tertiary Care Hospital- A 4-Year Review"

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Abstract

Aim: The aim of this study was to detailed analysis of the donor corneal tissue using Eye Bank Specular Microscope (EBSM). ***Materials and Methods:*** Retrospective analysis was done for 408 donor corneas procured and processed between April 2016 and March 2020, at an Eye Bank in a Tertiary Care Hospital for Endothelial cell density (ECD), coefficient of variance (CV), and percentage of hexagonality (6A) and were compared across different age groups & gender of the donor tissue. ***Results:*** The mean age of the donors was 66.78 ± 17.27 (range 12–95 years). The mean ECD of the donor cornea was 1400.65 ± 627.01 cells/mm2; the average CV was 37.5 ± 18.2; and the average percentages of hexagonal cells were 44.9%. The cell count was significantly greater in the donor corneas in age groups of <20, 21-30 & 41-50 years when compared to rest of the age groups. There were no significant differences between the age groups on basis of CV and 6A. The ECD of the male donors (65%) was more than that of female donors (35%). ***Conclusion:*** ECD is significantly greater in younger age group as compared to older age groups; however, there were no significant differences in CV and 6A. Endothelial cell analysis by Eye Bank Specular Microscopy is a great tool for the grading of donor tissues, its subsequent utilization for corneal transplantation & prognostic value of the graft survival.

Keywords: Eye Bank Specular Microscope, Coefficient of variance, donor cornea, endothelial cell density, percentage of hexagonality (6A).

INTRODUCTION

Corneal endothelial cell density (ECD), as determined by specular microscopy, is a traditional measure of corneal health and is one of the factors that predict the outcome of penetrating keratoplasty [1-4]. Qualitative and quantitative evaluation of the donor corneal tissue is an important factor for donor corneal selection, as it affects the prognosis of the corneal graft postoperatively. The primary goal after corneal transplantation is the preservation of a clear corneal graft. Endothelial cell loss after penetrating keratoplasty is an ongoing process. The exact cause of postoperative cell loss is unknown but may be a result of donor or preservation factors, surgical stress, cellular interactions between the donor and recipient, immune reaction, normal or accelerated cellular aging, or glaucoma [5]. The endothelial cells can sustain their function and maintain corneal deturgescence and clarity even if their number is decreased by 80% [6]. This continuous decrease in endothelial cell density (ECD), and their concomitant increase in size and shape are accompanied by a decreased ability to maintain and restore pump and barrier function of the endothelium. A critical point is reached when endothelial function fails, and the cornea swells and becomes hazy. Thus, surgeons prefer donor corneas with a high preoperative ECD, to offset post-transplant cell loss so that this improves the probability of graft survival. The cells should be observed in vivo without disturbing the cornea. Eye bank specular microscopy (EBSM) is an excellent tool for examining, photographing, and quantitatively evaluating the corneal endothelium. Mattern et al. [7] studied how the judgment on transplant ability of the donor cornea, is affected by specular microscopy results. The examination of donor corneal endothelium before its transplantation was first performed by Hoefle et al. [8] in 1970. Others have successfully used similar techniques to screen donor eyes [9-11]. With the increased use of isolated corneal storage in preservation media at 4°C, methods for safely examining the donor endothelium during the storage process were developed [12-14]. The Konan Center method [15] of noncontact EBSM can accurately assess ECD based on a certain

number of contiguous cells. Eye bank-generated specular images from the donor corneas can then be analysed for ECD and its morphology. The purpose of our study is to carry out a detailed analysis of the endothelial cells and morphology of the donor corneal tissue at our eye bank using EBSM.

MATERIAL & METHODS

Eye bank data of 408 donor corneas procured and processed at an Eye Bank in Tertiary Care Hospital between April 2016 and March 2020 were retrospectively analysed.

Data on donor age, gender and Eye Bank Specular Microscopy results were recorded. The "Center method" of Konan specular microscope (Konan Eye Bank Kerato Analyzer, Konan Medical Inc., Japan) was used for morphometric analysis of the endothelial cells. The approval of Institutional Ethical Committee was obtained for this study. Donor corneal tissue received from different parts of the state and procured under Hospital Corneal Retrieval Programme (HRCP) were analysed and details, such as age, gender, cause-of-death and history of any diseases were noted. The tissue blood sample was screened for serum HIV, hepatitis B virus surface antigen, serum venereal disease research laboratory, and hepatitis C. The corneo-scleral button of the donor eye was subjected to gross examination and slit-lamp bio microscopy for grading. When found suitable for keratoplasty, the corneo-scleral rim was preserved under strict aseptic conditions, appropriately labelled and stored in McCarey-Kaufman media (obtained from Dr. RP Centre for Ophthalmic Sciences – AIIMS, New Delhi) at 4°C.

Endothelial photographs were taken by the same resident using the EBSM. For each donor cornea, the indicators for quantitative analysis used were the mean ECD and standard deviation (SD) of the mean cell area; while cell morphology was indicated by the coefficient of variance (CV) and percentage of hexagonal cells (6A). ECD, hexagonality ratio (6A), and CV were then analysed with regard to the age and gender of the donor corneal tissue.

RESULTS

In the present study, endothelial cell analysis was done in 408 donor corneas. Data were analysed using paired and unpaired t-test. $P < 0.05$ was considered to be statistically significant. The male donors were more 65% (n = 268) than female donors 35% (n = 140). The mean age of the donors was 66.78 ± 17.27 (range 12–95 years). Maximum number of donors was in 71–80 years age group (27.45 %).

The mean ECD of the donor cornea was 1400.65 (±627.01) cells/mm2, the average CV was 36.02 (±17.03), and the average percentages of hexagonal cells were 47.5%. The cell count was significantly greater in the donor corneas in age groups of <20, 21-30 & 41-50 years when compared to rest of the age groups but the sample size was more in the 41-50 years age group as compared to the others (Table 1).

Table-1: Showing ECD, CV & Hexagonality in males and females among different age groups

Age groups (years)	Male / Female	Total	Endothelial Cell count (ECD)	Endothelial cell morphology	
				6A(Hexagonality)	CV
<20	3	4	2181.5 ±950.1	31.33 ±24.7	28.6 ±21.24
	1		2032 ± 277	42.5 ±3.5	41.5 ±2.5
21-30	3	4	2074 ± 341.7	52.5 ± 6.60	39.5 ± 12.6
	1		2424.5 ± 596.5	52.5 ±7.5	39 ± 13
31-40	10	15	1782.2 ± 634.18	48.1 ± 27.8	37.7 ± 14.4
	5		1754.7 ± 523.2	53.4 ± 26.6	34.1 ± 18.9
41-50	11	12	2166 ± 444.6	50 ± 14.2	40.14 ± 15.2
	1		2763.5 ± 186.5	33.5 ± 0.5	46.5 ± 11.5
51-60	17	27	1652.7 ± 644.4	43.8 ± 14.8	33.4 ± 14.13
	10		1212 ± 583.6	47.2 ± 24.2	29.7 ± 16.3
61-70	32	49	1190.1 ± 662.9	43.3 ±20.7	31.7 ± 18.9
	17		1391.4 ± 625.5	41.9 ± 17.4	41.4 ± 19.6
71-80	36	56	1222.3 ± 629.02	49.7 ± 25.2	33.5 ± 16.5
	20		853.9 ± 500.03	38.05 ± 25.5	31.4 ± 19.3
>80	23	40	928.3 ± 614.6	34.6 ± 23.9	28.9 ± 20.8
	17		917 ± 526.4	39.6 ± 23.6	31 ± 16.09
Average	135	207	1478.02 ± 621.9	47.8 ± 19.9	35.7 ± 16.02
	72		1229.1 ± 609.5	44.9 ± 21.1	37.5 ± 18.2

Average ECD of the male donors was more (1478.02 ± 621.9 mm2) than female donors (1229.1 ± 609.5 mm2), there was disparity in few groups as the female donors were less than male donors. (Figure 1) The ECD, CV, and 6A were not significantly different between both genders in different age groups.

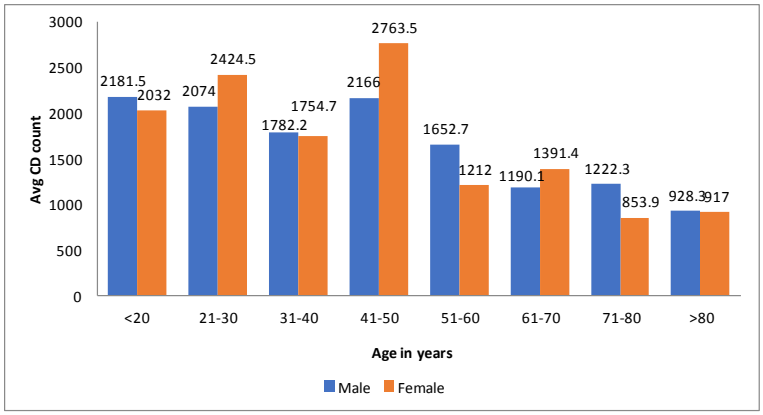

Fig-1: Showing ECD of male and female donors in different age groups

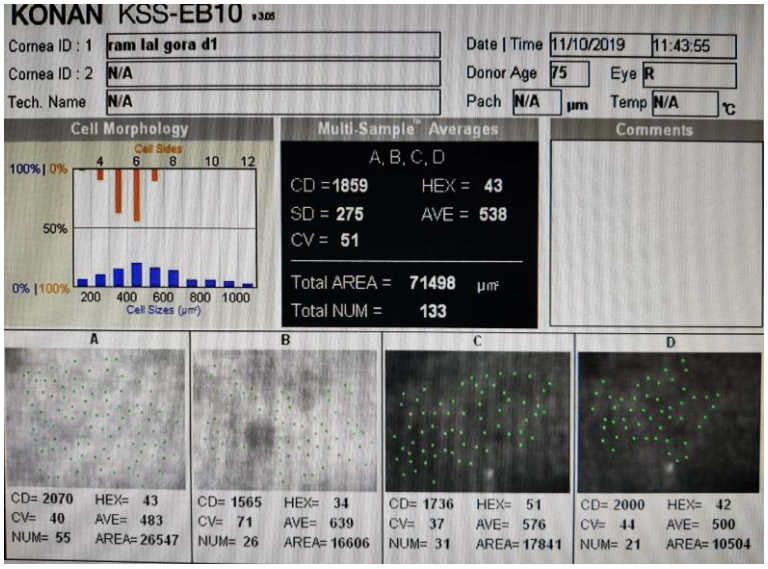

Fig-2: Showing EBSM of 75 year old male with average endothelial cell density

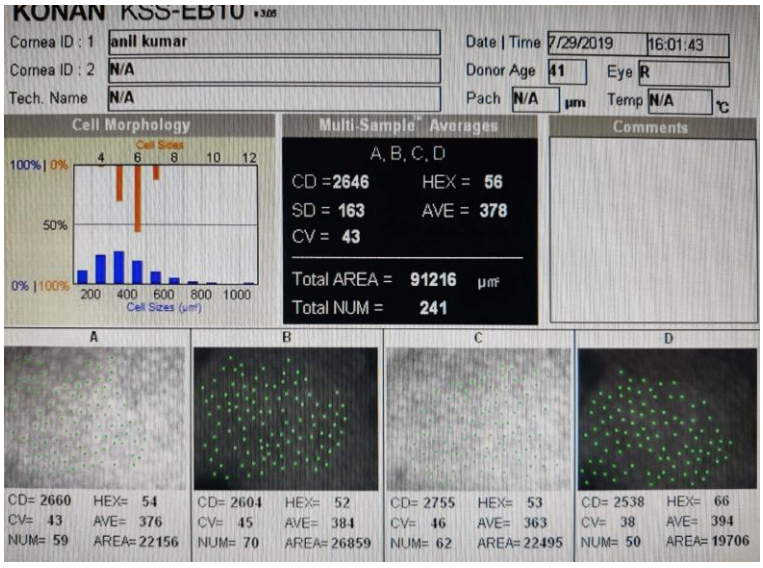

Fig-3: Showing EBSM of 41 year old male with good endothelial cell density

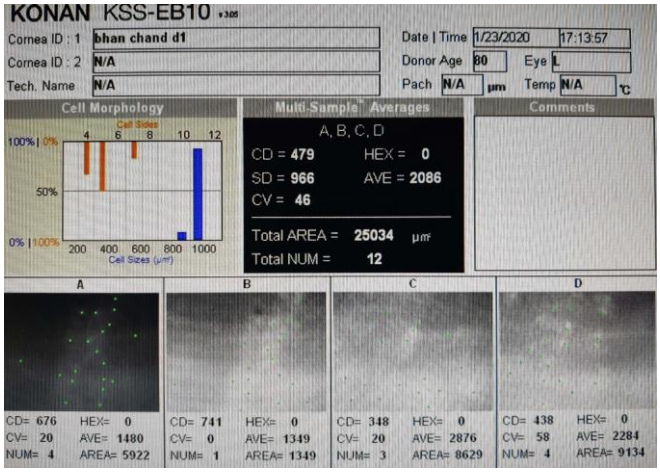

Fig-4: Showing EBSM of 80 year old male with poor endothelial cell density

DISCUSSION

Endothelial cell count is vital for the long-term survival of functional transparent graft. Qualitative morphometric analysis of eye bank specular images helps in the evaluation of the endothelium to assess the risks of intraocular surgery and prognosis of corneal transplantation. Abnormal endothelial structure can be identified by qualitative cellular and according to the number or size of the abnormal structures present or on the basis of an overall assessment of endothelial appearance, the donor tissue is graded and used for various indications of corneal transplant accordingly. Thus, Eye Bank Specular Microscopy helps in proper selection of donor tissues which finally affects the surgical outcome of the surgery. In our study, maximum number of donors was from 71 to 80 years group (27.45%) [Table 1]. The donor age distribution in similar studies showed that the majority of the donors were over 60 years of age, with a large group being between 70 and 80 years age group which is similar to our study [16, 17]. However, in the study by Gupta AR et al., the maximum number of donors was in the age group of 61-70 years [18].

In our study the mean age of donors was 66.78 ± 17.27 years, which was similar to the studies by Dasar LV et al., Patel HY et al. & Gupta AR et al. However, in the study by Ranjan et al., the mean age of donors was 52 ± 21 years that is less as compared to the participants in our study, and the majority of the donors were between 41 and 50 years age group [19].

Morphological assessment of the endothelial cells is a generally accepted method of evaluating the suitability of donor corneas for penetrating keratoplasty [20]. In our study, the mean endothelial cell count of the donor cornea was 1400.65 (±627.01) cells/mm2, the average CV was 36.02 (±17.03), and the average percentages of hexagonal cells were 47.5% & the ECD of the male donors was more (1478.02 ± 621.9 mm2) than female donors (1229.1 ± 609.5 mm2) which were not significantly different. The mean ECD of donor corneal tissue in a study by Ranjan et al. was 2857 (±551) cells/mm2, with majority (68%) of corneal tissues having ECD >2500 cells/mm2. Patel et al. found the mean ECD of transplanted corneas to be 3024 (±324) cells/mm2 [17]. In a study by Jorgen et al., the mean ECD of all the 946 eyes was 2708 (±350) cells/mm2 [21].

In our study, donor tissue from donors in age groups <50 years of age had high ECD as compared to the older age groups which was same as other similar studies. Corneas obtained from donors of advanced age seems to be initially unacceptable for transplantation, but specular microscopy helps in the final grading of donor corneal tissue. Similar to our study, Patel et al. also found a significant correlation between advancing donor age and lower ECD, where in age group <20 years the mean ECD was 3175 cells/mm2 while in age group of >81 years the mean ECD was 2917 cells/mm2. In a study by Tufekci et al., the mean ECD was 2884.0 (±457.3), mean SD was 112.7 (±36.2), mean CV was 31.4 (±6.7), and mean hexagonality was 61.1 (±8.6) [22]. The ECD was significantly higher in the first age group (5-19 years) compared to rest of the age groups while findings of CV and hexagonality did not show any age-based differences. In our study also, there is a similar finding. The mean ECD in our study was less as compared to other similar studies because 71% of the donor corneal tissues procured were from age groups more than 61 years.

Jadeja et al. and Chuw et al. concluded in their studies that large numbers of corneas from donors more than 65 years can be used successfully, to restore vision by revising the grading of the cornea after quantitative and qualitative assessment by specular microscopy [23, 24]. Our study also concludes the same for tissues from donors more than 60 years age. In our study, the corneas of donors above 80 years also got upgraded post-EBSM and there were favourable post-operative outcomes.

CONCLUSION

ECD is significantly greater in younger age group as compared to older age groups; however, there are no significant differences in CV and 6A. Male donors have more ECD than female; however, the difference is not significant. In our study, the donor tissues were either upgraded or downgraded based on EBSM. Specular microscopy is a vital tool for evaluation of the donor tissues & proper selection for different indications of corneal transplantation that also increases the chances of utilization of donor tissues for transplantation. Thus, endothelial cell analysis by EBSM alters the final grading of tissues and its subsequent utilization for corneal transplantation with good prognosis for graft survival.

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