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AGE BASED SPECULAR MICROSCOPIC EVALUATION OF FIRST 50 CORNEAL TISSUE OBTAINED THROUGH HCRP AT A NEWLY ESTABLISHED EYE BANK IN EASTERN INDIA

KEY WORDS: Corneo-scleral tissue, specular microscope, endothelial cell density, coefficient of variance, hexagonality.

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ABSTRACT

Background – Cornea transplantation has an important role in reducing the burden of corneal blindness but availability of corneal donor tissue is a significant limitation. The overall utilization of available cornea is further limited by the quality of donor corneal tissue. Specular microscopic evaluation of donor cornea is emerging as an indispensable tool of endothelial evaluation. **Aim –** Aim of this study to do an age based specular microscopic evaluation of first 50 corneal tissue obtained through hospital cornea retrieval program at a newly established eye bank in Eastern India **Material and method –** Total 50 corneo-scleral donor tissue were included in study after excluding the contraindication of cornea donation. Three tissues were could be not included in final assessment due to unavailability of specular microscopic image. 47 tissues were divided in different age groups – <20 years (10 tissues), 20-40 years (10 tissues), 40-60 years (10 tissue) an >60 years (17 tissues). Specular microscopic evaluation was done with Konan eye bank keratoanlyser - CellChek® D® (Konan Medical, Irvine, CA). Endothelial cell density (ECD), corneal thickness (CT), coefficient of variance (CV) and percentage of hexagonal cells (6A) were evaluated and correlated with age. **Result –** The average age of donor was 47.02 years. The average CT, ECD, CV and 6A were 523.42 +/- 16.37 micron, 2222.87 +/- 514.62 cells/mm2, 35.27 +/- 4.04, 53.06 +/-8.30 % respectively. The difference in ECD was significantly high in age group <20 years than in comparison to those between 20-40 years (p value - 0.018), 40-60 years (p value - 0.003) and more than 60 years (p value - 0.0001). There was a progressive decrease in ECD count with age but the change in ECD count between age group 20-40years and 40-60 years was not significant (p value - 0.12). ECD was significantly low in age group > 60 years in comparison to all other group i.e.<20 years (p value – 0.0001), 20-40 years (p value - 0.003) and 40-60 years (p value - 0.016). There was no significant difference in any other parameter (CT, CV, 6A) in any of the age group. **Conclusion -** Eye bank specular microscope is an important tool in proper evaluation of donor corneo-scleral button. The ECD is significantly high in tissue from younger age of donor with a progressive decrease in age. CCT, CV and 6A are found to have no correlation with age.

Introduction –

Corneal pathologies contribute a significant proportion of blindness in our society. The recent report on community eye care in India has reported corneal opacities as the second leading cause of blindness[1]. Corneal blindness is responsible for vision less than 6/60 in at least one eye in 6.8 million population, and of these, about 1 million have bilateral corneal blindness[2]. Cornea transplantation is the only surgical mean to combat corneal blindness. Modern microsurgical technique of cornea transplantation has evolved immensely in last two decades however there is still a limitation of availability of donor corneal tissue. A global survey focussed on demand and supply of corneal transplant has reported that about 53% of the world's population has no access to corneal transplantation and only one cornea is available for 70 recipients in need of it[3]. Voluntary donation of cornea still has not become a routine protocol due to lack of awareness, objection by family members, unsuitability to donate because of health issues and the unacceptable idea of separating the eye from the body[4]. Moreover, utilization of corneal tissue available through voluntary donation, has been reported to get further restricted due to medical history of donor and poor quality of corneal tissue. Singh et al in their study published on quality and utility rate of corneal tissue for the period of 2012 – 2015, reported corneal utility rate from the donors more than 70 years of age as only 27.6% with average optical purpose use of 9.74%[5]. A study published from a tertiary care trauma centre in India assessed the performance of existing eye procurement activities in trauma related deaths, who can serve as a large pool for potential tissue donors. The study reported that the overall successful eye procurement rate among the 1066 eligible trauma-related deaths was 1.9%. The most important reason (77.9%) behind this low success rate of cornea donation was 'lost opportunity' (difference between the total number of cases that were eligible for donation and the number of families approached for counselling). They advocated the need to augment existing administrative and manpower resources to increase the corneal procurement rate in the Indian population[6]. Jadeja et al has also emphasized on providing thoroughly trained, well-equipped enucleation teams, who can reach out to all suitable donors from hospitals and voluntary donors, for increasing the numbers of surgically competent tissues[7]. The lack of public drive towards voluntary donation and decreased utilization rate of corneal tissue available through voluntary donation lead to formulation of Hospital Cornea Retrieval Programme (HCRP), a dedicated, focussed and more controlled approach to tackle the above two problems. It is playing a pivotal role in availability of donor corneal tissue both in terms of quality and quantity. The utility rate of available corneal tissue has been further enhanced by the procedural shift in cornea transplantation from full thickness penetrating keratoplasty to lamellar keratoplasty. The selection criteria for certain type of lamellar keratoplasty can be less stringent than penetrating keratoplasty, allowing better utilization of available corneal tissue. The non-invasive qualitative and quantitative assessment of corneal endothelium is possible with specular microscopy. It is increasingly becoming an integral part of preoperative work up, to decide about quality of tissue and its suitability for the given surgical procedure. Gogia et al have reported an increased utilization of non-optical grade cornea and overall

increase in lamellar corneal procedures for any clinical grade of cornea with time. According to their study, the percentage of donor corneal utilization increased significantly over time with the rate being 65.08%, 70.06%, and 68.29%, respectively, in the years 2003, 2008, and 2011 (p value - 0.014). This increase in utilization was reported for therapeutic purpose but not for optical purpose (p value - 0.08)[8].

This study aims at age based specular microscopic evaluation of first fifty corneal tissue obtained through HCRP at a newly established eye bank in Eastern India.

Material and method –

This study was conducted at PMCH eye bank, a newly established eye bank associated with Patna medical college and hospital, Patna, a tertiary health care centre. This study was conducted adhering to the tenets of the Declaration of Helsinki after taking the departmental ethical committee clearance.

The first fifty consecutive corneal tissues obtained through HCRP, from December 2018 to February 2020, were included in the study. Corneal tissues from donor of unknown cause of death, on ventilation before death for more than 72 hours, septicaemia, malignancy and viral illness, seropositive for either HIV, hepatitis B, C and syphilis were not included in study.

Cornea retrieval was done within 6 hours of death, by in situ corneo-scleral button excision, with 2-3 mm of scleral rim. The corneal tissue was directly stored in Cornisol cornea storage media (Aurolab) at temperature of 4 degree centigrade. The specular microscopic analysis was done on day 1 of corneal retrieval, by a single trained person using Konan eye bank keratoanlyser - CellChek® D® (Konan Medical, Irvine, CA). It is a wide field ex vivo dual imaging specular microscope allowing a larger viewing field (1000 x 750 µm) with dual CMOS camera. It has an additional pachymeter associated with it, giving corneal thickness data. The specular microscopic evaluation was done following the 'centre method'. The quantitative and qualitative parameters assessed in this morphometric analysis were – Corneal thickness (CT), mean endothelial cell density (ECD), coefficient of variance (CV) denoting polymegathism and percentage of hexagonality (6A) denoting pleomorphism.
The ECD (Cells/mm^2) was determined from average cell area by the formula – 10^6 / average cell area. Polymegathism refers to variation in cell shape causing a decrease in accuracy of average cell area determination and ECD. It is calculated by the formula – Standard deviation in cell area / mean cell area, um^2. Pleomorphism or variation in cell shape (6A) or deviation from normal hexagonal morphology is described as percentage. The normal cornea is expected to have 60% hexagonal cells. Stress to corneal endothelium decreases this percentage.

These parameters were correlated with age of donor and the statistical analysis was done using unpaired t-test. P value less than 0.05 was considered statistically significant for this study.

Results –

The study included the first fifty corneo-scleral tissue obtained through HCRP at PMCH eye bank, a newly established eye bank in Eastern India.

The 50 tissues included in this study were divided in these age groups – less than 20 years, 20- 40 years, 40-60 years and more than 60 years. Out of these fifty tissues, three tissues could not be included in study as the specular microscopic evaluation was not possible due to poor quality of tissue.

The numeric distribution of tissue in different age groups is as follows (Table - 1) -

Table – 1 – Tissue distribution in different age group of donors

Age group of donors	Number of tissues
< 20 years	10
20- 40 years	10
40- 60 years	10
>60 years	17

The average age of donor was 47.02 years. The average CT, ECD, CV and 6A were 523.42 +/- 16.37 micron, 2222.87 +/- 514.62 cells/mm2, 35.27 +/- 4.04, 53.06 +/-8.30 % respectively.

The CT, ECD, CV and 6A in different age groups were as follows (Table 2) –

Table – 2 – Various specular parameters in different age group of donor corneal tissue

Age group	Average age	CT* (micron)	ECD** Cells/mm^2	CV***	6A**** (%)
<20 years	14.6 years	524.5+/-21.64	2800.7+/-511.64	35+/-2.44	56.7+/-9.92
20 – 40 years	29.60 years	533.1+/-16.25	2312.5+/-257.25	35.6+/-3.47	53+/-5.18
40- 60 years	53. 80 years	517.5+/-9.10	2169.7+/-90.78	34.7+/-2.31	50+/-3.65
>60 years	72. 35 years	520.58+/-14.92	1861.52+/-467.02	35.58+/-5.75	52.76+/-10.29

* CT – Corneal thickness, **ECD – Endothelial cell density, ***CV- Coefficient of variance ****6A - Percentage of hexagonal cells (6A)

The ECD was highest in the age group <20 years. The difference was significantly high in age group <20 years than in comparison to those between 20-40 years (p value - 0.018), 40-60 years (p value - 0.003) and more than 60 years (p value - 0.0001). There was a progressive decrease in ECD count with age but the change in ECD count between age group 20-40years and 40-60 years was not significant (p value - 0.12). ECD was significantly low in age group > 60 years in comparison to all other group i.e.<20 years (p value – 0.0001), 20-40 years (p value - 0.003) and 40-60 years (p value - 0.016). There was no significant difference in any other parameter (CT, CV, 6A) in any of the age group.

Discussion –

Cornea transplantation is the most viable option for visual rehabilitation of cornea blind population. Based on the present availability of safe donor eyes and utilization rates, it is estimated that 270 000 donor eyes are required to perform 100 000 corneal transplants per year in India, an approximately 4-fold increase from the present availability of donor eyes[9]. Although there is growing awareness towards eye donation pledging but the gap between requirement and availability of corneal tissue, is still huge. Grading of available corneal tissue is further significantly affected by death enucleation time, death preservation time and mode of tissue procured as reported by Raj et al[10]. HCRP was started in India in the year 1990 and has been proven itself as one of the most successful drive started by Eye bank association of India. It allows accessibility to a greater number of suitable donors, an opportunity to grief counsellor for motivating people toward eye donation, availability of medical history and blood sample of donor and controlled retrieval of corneal tissue within the recommended time period.

Specular microscope was invented by David Maurice in the 1960s but its use as a tool to evaluate the corneal endothelium started in 1970s by Bourne and Kaufman[11]. The CellChek® D® (Konan Medical, Irvine, CA), used in this study has a noncontact endothelial Dual CMOS camera with an imaging

field of 0.1mm^2. It provides a magnified view of donor corneal endothelium and a mean to evaluate its qualitative and quantitative property. The important parameters considered in endothelial evaluation are ECD, CV and 6A. The Eye Bank Association of America Medical Standard has considered specular microscopy as a useful tool for screening donor tissue to determine suitability for transplantation and adopted ECD determination as a medical standard in the year 2001[12]. Highlighting the utility of eye bank specular microscope, Jadeja et al in their article recommended to make it a mandatory tool for donor corneal tissue analysis[13]. According to this study, the final grading of corneal tissues get significantly altered when specular microscopic parameters are used in tissue quality grading. This can increase the subsequent utilization, an important step in decreasing the corneal blindness.

A study comparing demographic, clinical, microbiological, and utility profile of the corneas obtained through HCRP and VED program at National eye bank, India has reported that the average age of donor is lower in HCRP group[14]. In our study, although maximum number of donors belong to >60 years of age group, the average age of donor was 47.02 years. The average age of HCRP donor reported in study by Bajracharya L et al is 48.1 years which is similar to our study[15].

Although cornea donor study has reported that corneal graft survival on long term follow up is not directly related to ECD and donor age is not a factor in survival of most penetrating keratoplasties for endothelial disease[16], other studies have reported a slightly greater endothelial cell loss in tissues with older donor age[17,18].

The average ECD in our study is 2222.87 +/- 514.62 cells/mm^2. This is lower than the other studies published from India. A large study published from another eye bank of Eastern India has reported the average ECD of 2857 ± 551 cells/mm^2)[19]. Another study published by Gupta et al[20] have reported the mean endothelial cell count of the donor cornea as 2708.93 (±271.52) cells/mm^2. Patel et al. found the mean ECD of transplanted corneas to be 3024 (±324) cells/mm^2, a review study published from New Zealand national eye bank[21].

There was a progressive decrease in ECD with advancing age of donor age group. It was significantly higher in age group <20 years (2800.7+/- 511.64) than in comparison to other age groups. There was a decrease in ECD between age group 20-40years and 40-60 years, but this difference was not significant (p value – 0.125). Beyond 60 years of donor age again there was a significant decrease in ECD of corneo-scleral button. Other studies also have reported an age associated decrease in ECD. The reported ECD in donor age group of <20 years is 3193.00 (±341.30) by Gupta et al[20] and 3175 cells/mm^2 by Patel et al[21]. This difference can be explained partially due to limited number of corneal tissues evaluated in this study.

Although the ECD Studies based on changes in other endothelial parameters with age have published conflicting results. Galgauskas S et al[22] have reported higher ECD in younger population, weak correlation of decrease in CT with age and no correlation of variation in cell size and percentage of regular hexagonal cells with age in normal population. An Indian study documenting the normative database for Indian population, has reported that with increasing age, there is a statistically significant decrease in endothelial cell density ($p < 0.001$, correlation -0.387) and percentage of hexagonal cells ($p = 0.01$, correlation -0.127) and an increase in CV ($p = 0.02$, correlation 0.096)[23].

In our study the average CT, CV and 6A were 523.42+/- 16.37 micron, 35.27 +/- 4.04 and 53.06 +/- 8.30% respectively. Gupta et al in their study based on evaluation of 100 corneo-scleral donor tissue, have reported the average CV and 6A as 28.37 (±6.3) and 63.85% respectively.[20] These parameters (CT, CV, 6A) in our study were found to have no correlation with age of donor. Similar finding has been reported by Gupta et al[20] and Tufekci et al[24].

Conclusion – This study highlights the importance of eye bank specular microscope in proper evaluation of donor corneo-scleral button. The ECD is significantly high in tissue from younger age of donor with a progressive decrease in age. Other endothelial parameters like CV, CT and 6A have no correlation with age. With growing popularity and ease with lamellar corneal transplantation, specular microscopic evaluation of donor corneal tissue can help in proper selection of tissue and increase the overall utilization of donated cornea. Many donor corneal tissues with lower ECD can be utilized for anterior lamellar surgical procedure.

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