Original Research Article

A study to demonstrate importance of eye bank specular microscope to improve donor corneal tissue utilization

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Abstract

Aims: Evaluation of donor cornea by gross and slit lamp examination and by eye bank specular microscope and compare utilization based on findings. **Material and Methods:** Ours was a prospective study of 30 donor corneas which were evaluated by slit lamp biomicroscopy and after preservation in MK media, evaluated using eye bank specular microscope. Final decision of transplantation was made on grading of specular microscopy. We have analyzed whether eye bank specular usage can improve utilization of available tissue or not. **Results:** With slit lamp examination grading of donor tissue, excellent tissue was seen in 13.33%, 16.67%, 3.33% in age group of <20 years, 20-40 years and 40-60 years respectively while none of the tissue of 60-80 years show excellent grading. According to endothelial cell count by specular microscope, count of > 3000 cells/mm2 was seen in 16.66%, 36.67%,6.67%, 6.66% in age group of <20 years, 20-40 years, 40-60 years and 60-80 years. In tissues having death to preservation interval less than 8 hours, mean CD was 3335.5 cells/mm2 while tissues having DTPT more than 8 hours, mean CD was 2632.7 cells/mm2. **Conclusions:** With the use of eyebank specular analyzer, we can evaluate donor tissue thoroughly thus improving untilization of available tissue as well as improve outcome of keratoplasty

Key Word: eye donation, endothelial count, eye bank specular analyzer.

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INTRODUCTION

World Health Organization (WHO) has conducted a survey in which they found that every 5 seconds, someone goes blind[1]. In the world, there are 45 million blind people and this number increases by 1-2 million every year[2]. Out of total causes of blindness, corneal blindness is the second most common cause of preventable as well as treatable blindness in India, and it accounts for about 6.8 million of the total blind cases in the world. There are 1,20,000 corneal blind patients with 25000 to 30000 patients added every year in India according to survey of National Programme for Control of Blindness. In India number of patients with unilateral corneal blindness will increase to 10.6 million by 2020[3]. 90% of the global cases of ocular trauma and corneal ulceration leading to corneal blindness occur in developing countries. Mainstay of treatment in such condition is corneal transplant. In India, donor eye collection is around 22000 eyes every year, which is far less than the requirement[4]. To increase the supply, eye donation awareness is must. With the proper assessment and utilization of retrieved tissue, we can fill a small gap in demand supply ratio. Eye bank specular microscope can evaluate endothelial cells accurately thus tissue which was not good for transplant according to slit lamp examination can also be used if specular shows good endothelial status, thus improving utilization as well as outcome of transplant surgery.

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MATERIAL AND METHODS

Ours was a prospective study in which we studied data of 30 donor corneal tissues with age group of 5-85 years, in which through slit lamp as well as eye bank specular assessment was done. Donor cornea with age less than 5 years and more than 85 years were excluded from study. Donor corneas with history of death due to HIV positive, HBsAg positive, metabolic disorders, infective etiology, malignancy and viral illness were excluded from the study. Name, age of donor, time and cause of death, death to enucleation interval, death to preservation interval, method of preservation, clinical condition of the donor eye and endothelial assessment was recorded and evaluated. Corneal evaluation begins with a gross optically unaided examination of the corneas. A simple penlight examination can reveal epithelial defects (drying, erosion, sloughing), corneal edema with associated haze and striations due to folding of descemet's membrane, abnormal corneal shape, blood or cloudiness in the anterior chamber, arcus senilis, corneal scars or infiltrates. Whole eyes are examined by the slit lamp within the eye jar. The first part of the examination is a low to medium power examination of the total cornea at approximately 20˚-30˚ angle of incidence and moved to scan the entire cornea. The epithelium is inspected for integrity and overall condition specifically abrasions, defects and foreign bodies. Penetration of bowman's membrane by foreign bodies or by epithelial defects should be ruled out. The stroma is examined for overall clarity, amount of edema and stromal folding. Using higher magnification and a thin slit can better characterize the opacity as scarring or inflammatory infiltrates. Stromal folds are associated with corneal oedema. Folding of descemet's membrane can be most easily observed by directing a relatively narrow slit with 30˚-40˚ of incidence on the mid to peripheral cornea and focusing on the endothelial reflex at low to medium magnification. A clinical grade ranging from excellent to poor was assigned (Table 1)[5,6]. After the globes were received in the eye bank, all aseptic precautions were taken. The globes were irrigated with betadine solution (5%) and antibiotic drops. The globe was then wrapped with sterile gauze and secured with a hemostat. Conjunctival scissors then used to cut away any conjunctiva from the limbus. A sharp-pointed scalpel blade was used to incise the sclera, 2-3mm. posterior to the limbus without penetrating the choroid. With a curve tipped scissors, the sclera was then cut around the circumference of the scleral rim. Care was taken not to penetrate and cause a vitreous leak or physically damage the cornea while completing the incision. Using a toothed forceps to hold the corneoscleral rim and the ciliary body, choroid and other posterior structures were very carefully pushed away and separated from the corneoscleral rim at the scleral spur. Extreme care was taken not to bend, fold or stretch (stress) the cornea. The corneoscleral button obtained was placed in a sterile container filled with M-K media. The tissue was appropriately labelled and stored in MK medium at 4˚C. Endothelial cell count and morphological analysis of donor cornea was done using Konan Eye bank keratoanalyser. For the best observation of the corneal endothelium, the cornea must be at room temperature. Thus, the most convenient time to perform keratoanalysis after the placement of the cornea in storage media, allowing sufficient time for the media to equilibrate with room temperature and for deturgescence of the cornea. Corneas can also be observed after a period of 4ºC storage and subsequent warming to room temperature. The cycle of cooling, warming and re-cooling (freeze thaw cycle) of a donor cornea has been shown to have no adverse effects on the metabolic or morphometric status of the donor cornea. The basic procedure was to place the media bottle containing the preserved cornea on the microscope stage (the holder for the bottle). The bottle was then positioned to make the light from the microscope shine on the central corneal endothelium to get the maximum reflected image. Using the microscope stage, the X-Y-Z plane position was also adjusted to maximize the reflected image intensity. The image was focused and the area scanned to find a representative group of cells. The morphology of endothelial cells was observed and presence of any pathology such as guttate, folds, snail tracks, etc. were looked for at the same time. The 'Center method' of Konan eye bank keratoanalyzer was used for obtaining the endothelial cell density and the parameters get displayed automatically.

OBSERVATION AND RESULTS

The large number of cases were in the age group of 21-30 years and 61-80 years i.e.36.67 and 23.33% respectively while minimum number of cases were in age group of 5-10 years i.e. 3.33% and 51-60 years i.e. 3.33% cases. which was comparable to Szaflik *et al* study[7] which was done in 2003. Median age was 58years. While in szaflik *et al* study, large number of donor cases were seen in the age group of 61-80 years i.e.49.23 %. Minimum number of cases were in age group 5-10 years i.e. 3.33% cases as there is still less awareness about eye donation and its criteria. It was observed that there was male preponderance, 70% of the total cases which is comparable to P. Gain et. al study[8] which was done in 1997. In our study death to preservation interval was minimum 1.20 hours and maximum 19 hours with mean of 5.10 hours. It was observed that when death to preservation interval was <8 hrs, then donor cornea is

clear i.e. there was no epithelial defect in the cornea in 65.22% cases, while in 34.78% cases epithelial defect was present. When death to preservation interval was ≥8 hrs, 57.14% tissues does not have epithelial defects while 42.86% corneas have epithelial defect. The test of association between death to corneal button preservation interval and donor epithelial status has been applied to know the statistical significance. Cut off point for between death to preservation interval was arbitrarily kept at 8 hours and the donor corneal epithelial status which were mutually exclusive. P value – 0.476 (>0.05%) which was not significant due to smaller sample size. The rating of the morphological state of corneas suitable for PK depends mostly on the time between death and preservation and duration of preservation. Corneas obtained shortly after the donor's death showed higher endothelial cell density and better overall rating than those removed after a relatively longer period of time after the donor's death. In donor tissue of age group of <20 years, 13.33% tissues have excellent grading and 3.33% very good grading. Tissue of age group 20-40 years 16.67% tissue have excellent grading, 13% tissue have very good grading. While in age group of 40-60 years, 3.33% tissue have excellent, 3.33% have very good and 3.33% have good grading. In tissues of 60-80 years none tissue had excellent grading, 3.33% tissue have very good and 10% tissues have good grading. In our study, 33.33% tissue had excellent endothelial layer(figure 1), 53.34% had very good quality endothelial layer while only 13.33% tissue had good endothelial layer.

After specular biomicrocopic examination, endothelial count >3000 cells/mm^2 was seen in 16.66% donor corneas of age group <20 years, 36.67% donor corneas of age group 20-40 years, 6.67% in 40-60 years age group while 6.66% tissues of age group 60-80 years showed the same endothelial count. While without specular microscopic examination, none tissue show excellent grading while with specular examination this 6.66% tissue of age group 60-80 years showed endothelial count >3000 cells/mm^2. Thus we can improve the utilization of such tissues. Cell count of 2500-3000 cells/mm^2 was seen in 10% tissues of 20-40 years and 6.67% tissues of 60-80 years. 2000-2500 cells/mm^2 count was seen in 6.67% tissue of 60-80 years age group. 1500-2000 cells/mm^2 count was seen in 3.33% tissues of 40-60 years and 60-80 years each. In age group < 20 years, mean CD was 3742 cells/mm^2, mean SD was 92.6, mean CV was 36.4 and hexagonality of 66. While in age group of 20-40 years mean CD was 3280 cells/mm^2, mean SD was 94.80, mean CV was 32.86, mean hexagonality was 62. In age group of 40-60 years, mean CD was 2771 cells/mm^2, mean SD was 97.33, mean CV was 36.33 and mean hexagonality was 60.33. In the age group of 60-80 years, mean CD was 2683, mean SD was 104.2, mean CV was 35.14, mean hexagonality was 61.28. in tissues having death to preservation interval less than 8 hours, mean CD was 3335.5 cells/mm^2 while tissues having DTPT more than 8 hours, mean CD was 2632.7 cells/mm^2.

Table 1: Donor corneal tissue evaluation criteria

Grade	Description
Excellent	Endothelial cell count-3000 Cells/mm^2, No epithelial defects, Crystal clear stroma, No arcus senilis,No folds in descemet's membrane, Excellent endothelium-no defects
Very good	Endothelial cell count-2500 To 3000 Cells/mm^2, Slight epithelial haze/defects, Clear stroma, Very slight arcus, Few light folds in descemet's membrane, Very good to excellent endothelium-no defects
Good	Endothelial cell count-2000 To 2500 Cells/mm^2, Moderate epithelial defects, Light to moderate Cloudiness, Moderate arcus senilis <2.5mm, Numerous but shallow folds in descemet's membrane, Few vacuolated cells
Fair	Endothelial cell count-1500 To 2000 Cells/mm^2, Obvious epithelial defects >60%,Moderate to heavy stromal Cloudiness, Heavy arcus senilis >2.5mm,Heavy folds-numerous, deep, central, Fair to good to endothelium-Moderate endothelial defects, vacuolated cells, low cell density
Poor	Endothelial cell count- Less than 1500 Cells/mm^2, Moderate vacuolated cells, Severe stromal Cloudiness, Marked folds-heavy, numerous, central, Fair endothelium-Marked endothelial defects, numerous central vacuolated cells, low cell density, Technical problems in removal

DISCUSSION

Cadaver corneal tissue for transplantation must meet guidelines for acceptability which includes the serological tests and medical history of the donor. However, many potential donor corneas, usually from elderly donors are rejected for transplantation because of the lower endothelial cell count and possible age-related diseases. The availability of tissue is also affected by cultural, logistical, and technical difficulties. Transplant-grade donor graft material becomes unsuitable because of high postmortem time or damage occurred during the handling of these fragile donor corneas. These are some of the confounding problems that add to the global shortage of suitable transplant-grade cornea tissues. Age of donor, DTET and DTPT of donor eyes and the clinical grading, which have been often used by Eye Banks, are

not good guides for decision making regarding the quality of tissues and its utilization. The impact on donor tissue grading and its benefit, on the basis of corneal endothelial cells (Specular Microscopy) is well acceptable. Despite the clear evidence of importance of endothelial cells and the advantage of specular microscopic technique, there is lack of application of EBSM and rationalizing the distribution of tissue for different indications of corneal transplant surgeries based on it. As seen in our study, proper use of the quantitative and the qualitative criteria of the donor corneal endothelial cells as analyzed by EBSM leads to change in final grading and utilization of the tissues. Shortage of tissues suitable for surgery is a concern which is more relevant for developing countries and supplies of quality donor corneas continue to lag behind the demand despite of many activities. This shortage appears to be partly due to the custom in many centers to use tissues only from younger donors, tissues with very short DTET and DTPT, tissues with phakic status of eyes and those with excellent to good Torch Light Examination/ Bio microscopic grading. In reality, there is a sizeable pool of good quality tissues amongst the available tissues and with improved evaluation techniques, as seen in our study, it is possible to identify these tissues and to add them to the supply of available corneas. We are trying to focus on how to utilize more tissues from the available pool of tissues, thus optimizing utilization against discarding them for reasons which may not be indicative of their actual grade. Our study is clearly indicating that by performing EBSM, there can be a definite positive influence in optimized utilization of tissues from the available pool.

CONCLUSION

From our study, it is clear that eye bank specular examination can improve tissue utilization as well as improve outcome of surgery. We strongly recommend use of eye bank specular microscope in every eye bank to enhance quality of eye care.

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