

DONOR CORNEA ANALYSIS, WHOLE GLOBE ENUCLEATION VS. IN-SITU TECHNIQUERekha Gyanchand¹¹Cornea Consultant and Medical Director, Eye Bank, B. W. Lions Super Speciality Eye Hospital, Bangalore, Karnataka.**ABSTRACT****BACKGROUND**

The donated corneas are very precious & the method of retrieval is very important to maintain the integrity of the donor cornea.

Aim: To compare the enucleation & In-Situ (IS) technique of donor cornea retrieval. Does the retrieval technique have any bearing on the donor cornea tissue quality?

MATERIALS AND METHODS

In this study, 100 donor eyeballs were retrieved by enucleation & 100 corneoscleral rim excisions done. The corneal epithelial integrity, Descemet membrane folds, corneal endothelial cells analysed. The transplantation rate, DTE & DTP considered.

Study Type- Prospective Analysis.

RESULTS

The results were analysed in 2 groups depending on the type of retrieval- A & B. Each group was again subdivided into 1 & 2 depending upon time factor that is death to preservation of corneal tissue.

- Group A: Enucleation group. Sub-Group: A1 retrieval within 8 hours: n=73%, A2 retrieval more than 8 hours: n=27%
- Group B: In-situ group. Sub-Group: B1 retrieval within 8 hours: n=34%, B2 retrieval more than 8 hours: n=66%
- Group A Mean Epithelial Defect (ED): 74.49%, Significant DM folds 67.12%. Mean endothelial cell density range 2968.85-2983.82
- Group B Mean ED: 47.25%. Significant DM folds 47.55%. Mean Endothelial cell density range: 2975.5-3222.5
- Utilization rates: Group A 67%, group B 98.49%. Corneas un-fit for use in Group A 2.5% and Group B 1.19%.

CONCLUSION

In-situ technique corneal tissue quality is superior to whole-globe enucleation retrieval.

KEYWORDS

Death to Enucleation Time (DTE), Death to Preservation Time (DTP). Enucleation (EN), In-Situ (IS), Epithelial Defect (ED).

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BACKGROUND

The Tertiary Eye banks in cities do have their own Eye Retrieval centers (ERC) which are located in peripheral towns & rural villages. In these centers the donor eye retrieval is done either by enucleation or in -situ excision technique & this depends upon the training provided to the Eye bank Technicians. The Eye bank Technicians are non-medical personals trained in corneal recovery. In some centers the junior doctor or a family physician is also trained in enucleation Technique. Once the donor eyes or corneoscleral button are retrieved from this peripheral ERC it is transported to the parent eye bank for further processing & distribution. This study would help us to understand the best Technique of Donor Eye Ball Retrieval that would preserve the corneal integrity better. The other

factors that have an effect on the corneal quality are death-to-enucleation time (DTE),¹ and death-to-preservation time (DTP).²

In India & many other countries, whole globe Enucleation is still the most popular technique.³

MATERIALS AND METHODS

This is a prospective study done at Lions International Eye Bank, Bangalore.

100 donor eyes whole globe enucleation technique of retrieval was done & in 100 donor eye in-situ retrieval was done.

The whole globe retrieval was done by fellow doctors & the in-situ was done by trained eye bank technicians. The Technicians were trained in lab excision for a period of 1 year before they started in-situ excision.

The regular eye donation protocol was followed like – complete medical history, consent for eye donation from the legal next of kin & two witness consent.

Exclusion Criteria

Donor eyes were not collected from contraindicated cases.

Financial or Other, Competing Interest: None.

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Inclusion Criteria

The non - refrigerated eye donors, Retrieval of the eyes were done within 8 hrs. of death. In Refrigerated donors, the retrieval needs to be done within 12 hrs. of death & the donor must be refrigeration within 6 hrs. of death.

Post-retrival Protocol: Following the enucleation technique, the donor whole globe was transported by the moist chamber method to the eye bank. At the eye bank, lab-excision was done& preserved in Cornisol preservative media.

In case of in-situ excision the corneoscleral button was directly preserved in Cornisol media.

The igloo container was used for transportation of the retrieved tissue to the eye bank at 4°C with either methods of retrieval.

At the eye bank, Serology test was carried out for HIV, VDRL, and HBsAg & HCV. The donor tissue evaluation was done by slit lamp biomicroscope & specular microscope.

Statistical Methods

Data were recorded on a predesigned proforma and managed on an Excel spreadsheet. Quantitative variables were assessed for approximate normality and thereafter summarized as mean and SD. Student t-test was used to compare the mean values between the 2 groups.

RESULTS

The results were analysed in 2 groups depending on the retrieval method. Group A is the Enucleation technique & group B is the In-situ Technique. Each group was again subdivided into 1 & 2 depending upon death to preservation time (DTP).

Group A: Enucleation group.

Sub-Group: A₁ retrieval within 8 hours: n=73%, A₂ retrieval more than 8 hours: n=27%.

Group B: In-situ group.

Sub-group: B₁ retrieval within 8 hours: n=34% B₂ retrieval more than 8 hours: n=66%. The number of cases in the study group was 200 donor eyes. 100 eyes were retrieved by enucleation techniques & 100 by in-situ excision.

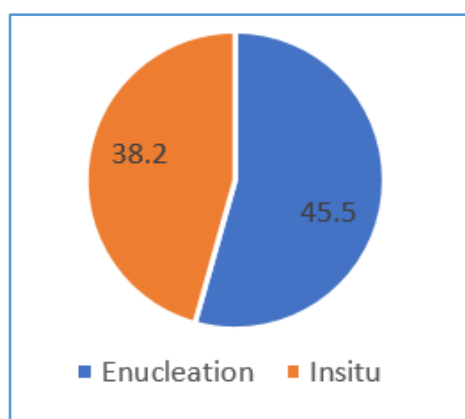


Table 1. Mean Age Distribution

The table 1 shows Mean age in the study group was 40.28 yrs. The mean age in the Enucleation group were slightly older 45.5 yrs., The In-situ group had younger donors with a mean age of 38.2 yrs.

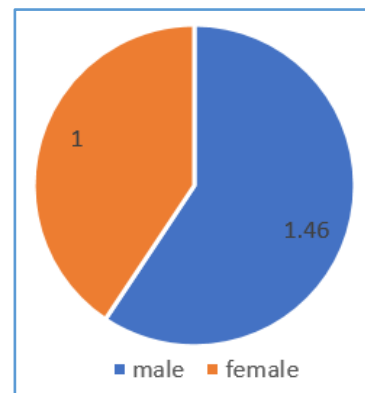


Table 2. Gender

Table 2 shows the Male to Female ratio & it was 1.46:1.

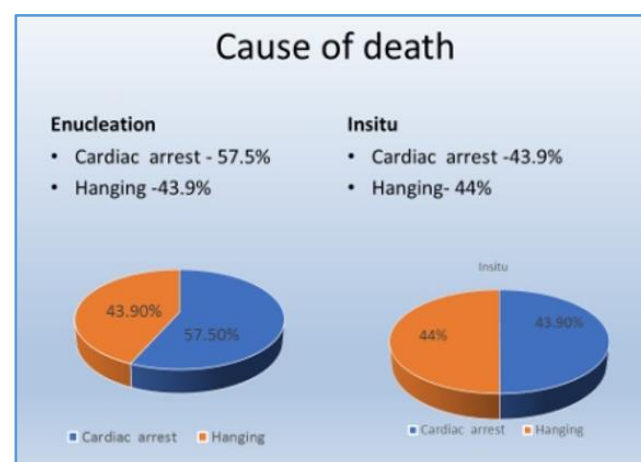
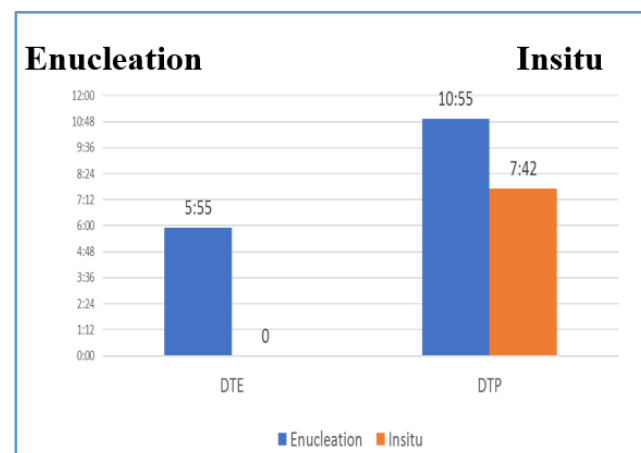


Table 3. Cause of Death

The table 3 shows the major cause of death in the enucleation group& in-situ group was the same, cardiac arrest & hanging with an incidence of the Cardiac arrest of 57.5% & Hanging 43.9% in Enucleation group& In-situ the incidence was 43.9% & 44%.



Tale 4. Death to Preservation

The table 4 shows the mean DTP in both the groups, the Enucleation group the Mean Death to Enucleation time (DTE) was 5 hrs. 55 min. The moist chamber was the method of preservation of the whole globe until it was transported to the eye bank. At the Eye bank, Lab excision was done & then preserved in Cornisol & the mean DTP was 10 hrs. 55 min.

In the In-situ group, the Mean Death to Preservation time was 7 hrs. 42 min. The corneoscleral rim was preserved in Cornisol preservative media.

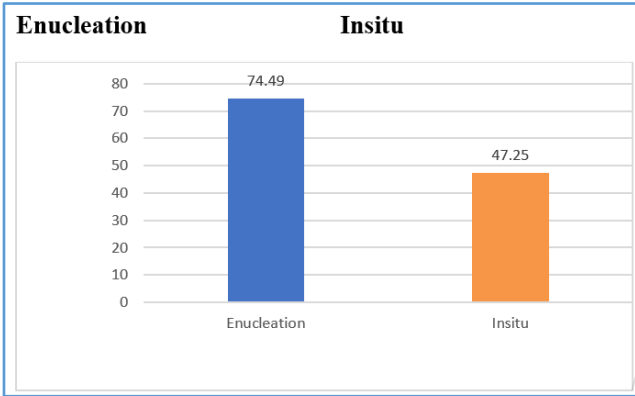


Table 5. Corneal Epithelium Changes

Table 5 shows the corneal epithelial changes in both the groups. The epithelial changes noted were epithelial sloughing. The mean epithelial sloughing in Enucleation group was 74.49%. In the in-situ group, the Mean Epithelium sloughing was 47.25%

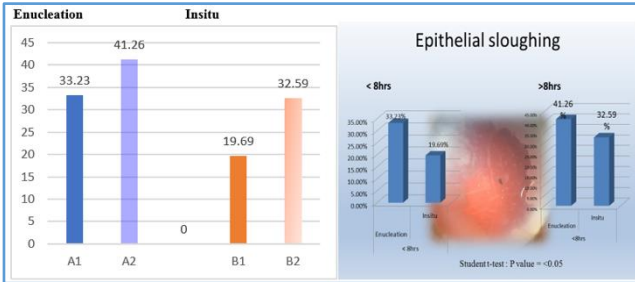


Table 6. The Epithelium Changes – Sloughing Vs. DTP

Enucleation method (A) In-Situ Method (B)
A1 Retrieval within 8 hrs. of death. B1
A2 Retrieval beyond 8 hrs. of death B2

The table 6 shows the epithelial sloughing was seen to be more in both the groups if retrieved was beyond 8 hrs. of death. Within 8 hrs. of death the epithelial integrity was maintained much better in In-situ it was 19.69%, compared to the enucleation group which was 33.23%.

Student t-test compared between both sets of variables showed: P = <0.05

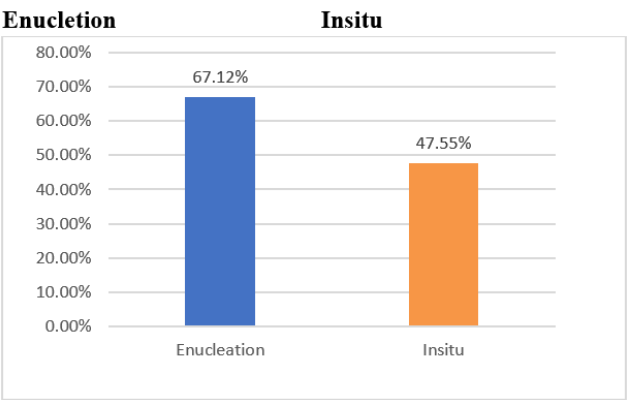


Table 7. Descemet's Membrane Folds

The figure 7 shows the Enucleation technique cornea has more DM folds 67.12% when compared to In-situ techniques which have 47.55%.

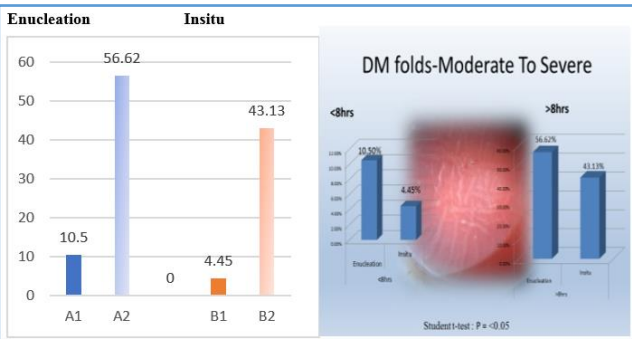


Table 8. Descemet's Membrane Folds Vs. DTP

The Table 8, DM folds were only 4.45% in- situ when compared to enucleation technique which was 10.5%. The preservation of the cornea in cornisol within 8hrs of death, less the DM folds.

The Descemet's membrane fold is significant in both the groups when retrieval is beyond 8hrs of death Enucleation group is 56.62% & in-situ group 43.13%.

Student t-test compared between both sets of variables showed: P = <0.

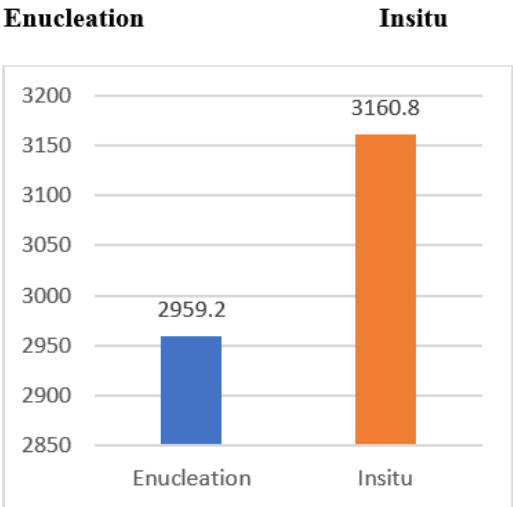


Table 9. Corneal Endothelial Cell Density

The table 9, shows the mean corneal endothelial cell density in the Enucleation group was 2976.2 cu.mm/ sq vs 3160 cu.mm/sq in-situ group.

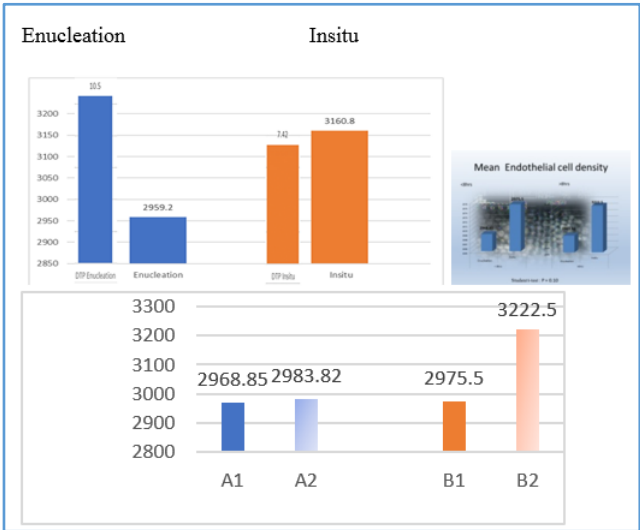


Table 10. Endothelial Cell Density vs. DPT

Table 10 shows the endothelial cell density & the DPT. The Time factor of less than 8hrs did not make a significant difference in both the groups. But more than 8hrs the endothelial cell density was better in the in-situ group. Student t-test compared between both sets of variables showed P =0.10 which is not statistically significant.

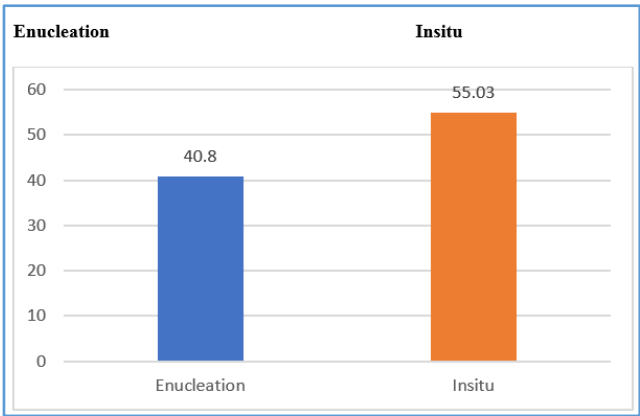


Table 11. Corneal Endothelial Hexagonal Cells or Index of Pleomorphism

The table 11 shows the percentage of endothelial hexagonal cells is preserved better in the in-situ group 55.03% when compared to the Enucleation group which is 40.8%.

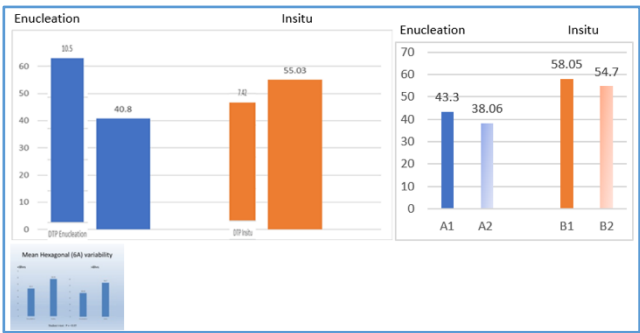


Table 12. Hexagonal Cells Vs. DTP

Table 12, (DTP=Death to preservation) shows the ratio of hexagonal cells vs DTP time. The mean DTP was prolonged in the enucleation group of 10.5hrs and the hexagonal cells were only 40.8%. In the in-situ group, the mean DTP time was 7.42 hrs. & the percentage of hexagonal cells was 54.70%. Student t-test was P = <0.05

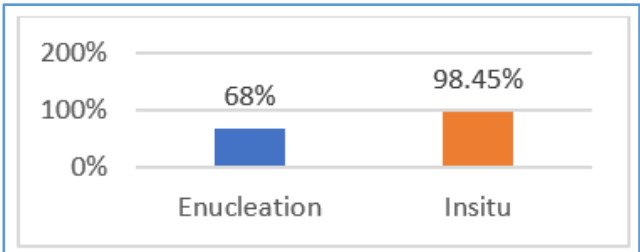


Table 13. Corneas Transplanted

The Table 13 shows the number of corneas utilized for optical transplantation was much higher in the in-situ group (98.45%) when compared to the enucleation group (68%).

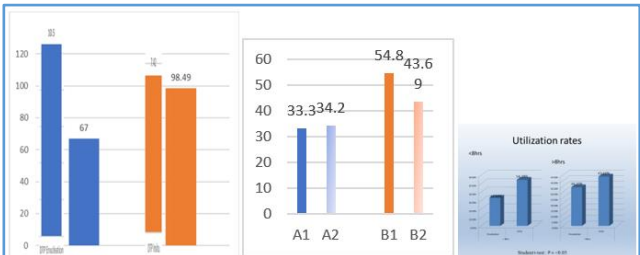


Table 14. Corneas Transplantation vs. DTP

The table 14 shows the DTP was higher in the enucleation group when compared to the in-situ group. The DTP in the enucleation group less than 8 hrs. or more than 8hrs the utilization of cornea for transplantation was the same 33.33% & 34.20%. In the in-situ group the utilization of cornea for transplantation was higher when the retrieval of tissue is less than 8hrs or more than 8hrs the utilization rate was much higher 54.80% & 43.96%. Student t-test was P = <0.05

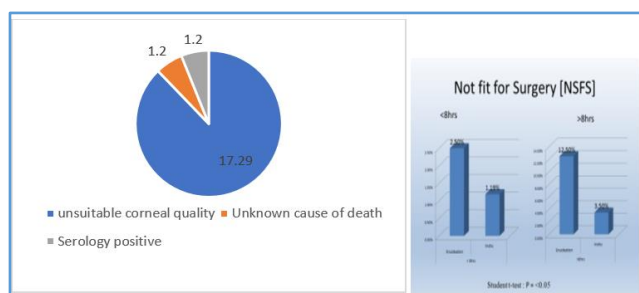


Table 15. Donor Corneas Not Suitable For Surgery (NSFS)

The Table 15 shows the donor corneas unfit for transplantation were due to the following reasons - serology positive in any of the tests VDRL, HIV, HBsAg, HCV, unknown cause of death, corneal infiltration & severe DM folds. In the enucleation group, 15% of the corneas were unfit for Transplantation & 4.69% of the corneas unfit for transplantation in the in-situ group. Student t-test was $P < 0.05$.

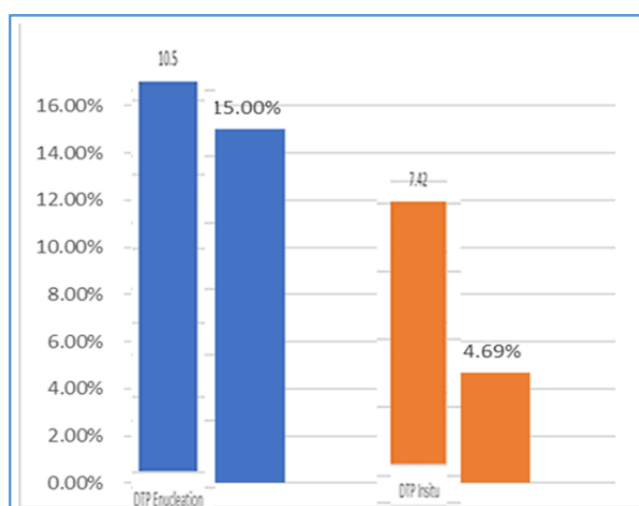


Table 16. DPT Vs. Not Suitable for Surgery

The table 16 shows the DPT was 10.50hrs in the enucleation group & 15% of the corneas were unsuitable for transplantation. In the in-situ group, death to preservation time was 7.42hrs only & only 4.67% of the corneas were unsuitable for utilization.

DISCUSSION

Shortage of quality donor corneas is the major contributing factors in INDIA for a low annual transplantation rate. The shortage of donor corneas is not only due to lack of public awareness, but it is also due to the lack of standard eye bank protocols. The elimination of corneal blindness demands co-ordination of all Eye Banking Techniques at each level of donor cornea retrieval & processing.

Eye Banking is progressing rapidly as a profession by itself, with a lot of non-medical personals getting involved in technical & non-technical aspects of eye banking.

In this study, we have analysed the² cornea retrieval techniques. This is to understand if retrieval techniques have a role in maintaining the donor cornea integrity.

The Mean age distribution in our study group showed younger donors in the in-situ group 32.2 years, A study done by Dan B. Rootman et al⁴ showed older donors in a mean age of 57.6 years.

In our study Male:female ratio was 1.46:1 and a similar result was found in root man et al – Male:Female 1.93:1

The common cause of death in both the groups was found to be Cardiac arrest 33.3% and hanging 44%. while study by root man et al showed cardiac arrest 24.6% and neoplasia 21.6% as two most common cause.

Mean Death to preservation time in our study was 10hrs:55min in enucleation and 7hrs: 42min in in-situ whereas in Jhansi et al⁵ study it was 9hrs in enucleation and 4hrs in-situ.

The corneal epithelial changes like the Epithelial sloughing in In-situ group were lesser (P -value < 0.05) compared to enucleation, which had similar results from a study by Jhanji et al⁵ and root man et al.

Our Study	Jhanji et al	Rootman et al
Enucleation: 33.3%	27.45%	33.9%
In-situ: 19.69%	12.8%	9.7%

Table 17

Descemet's membrane folds: DM folds were lesser in In-situ group (P value < 0.05) compared to enucleation group. which was similar to study by root man et al and study done by Grutzmacher RD et al⁶ This would again suggest that DM folds are more likely to occur in prolonged Death to preservation time.

Our Study- Mean Significant DM folds-	Grutzmacher RD et al	Rootman et al
Enucleation: 67.12%	13.7%	74.3%
In-situ: 47.55%	9.4%	40.9%

Table 18

Our Study- Mean ECD	Bohringer et al-Mean ECD
Enucleation: 2959.2	2603.8
In-situ: 3160.8	2587.7

Table 19

The Mean Endothelial Cell Density was similar in both the enucleation and In-situ groups (P -value > 0.05) which was similar to studies by Bohringer D et al.⁷

Researchers have suggested that in-situ procurement can lead to tissue trauma, manifesting as increased incidence and severity of endothelial cell loss. This study, using a much larger sample, did not replicate this finding. This study found no difference in cell counts between in situ and whole-globe harvested eyes and this result tends to agree with previous comparisons.

Our Study- Mean 6A	Matsuda et al- Mean 6A
Enucleation: 40.8%	45.7%
In-situ: 55.3%	52.3%

Table 20

But mean hexagonal variability was better in an in-situ group; 55.3 to 40.8 in-situ group compared to enucleation group where P value was >0.05 and was comparable to results by Matsuda et al.⁸

The number of corneas used for transplantation was higher in the Insitu group 98.4% compared to enucleation group 67.5% (P-value <0.05). In comparing results for that retrieved within 8hrs with a study done by Jhanji et al, similar results were obtained. This is due less DPT in the in-situ groups than enucleation group.

Our Study: Mean Transplantation Rate >8 hrs. of Retrieval	Jhanji et al
Enucleation: 33.3%	34.2%
Insitu: 54.8%	43.69%

Table 21

Our findings have shown that some of the differences in overall tissue quality between in-situ and whole-globe enucleation is due to DTP time. It appears that in -situ extraction may be the prime determinant of overall initial quality, although when considering all corneas, these eyes tend to be rated better overall.

In situ eyes tend to be placed in storage medium earlier than the whole-globe eyes, which may improve their overall initial quality.⁹

Moist chamber method of tissue harvesting can cause rapid autolysis of corneal endothelium & deterioration of cornea. The most important criticism against the moist-chamber storage method is that in an intact eye, the endothelium is exposed to postmortem changes in the aqueous humor.¹⁰

Our results show that the in-situ excision technique has better tissue quality & this could be due to a short DTP & a single procedure by which the tissue is preserved in the media.

CONCLUSION

Our data states that the in-situ excision is an effective, simple and safe method of donor cornea retrieval. The donor cornea quality preservation is excellent & utilization for transplantation is much higher. A change over from enucleation to in-situ excision is safe and feasible.

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